HYPOXIA, HABITATS AND COMPETITION: DETERMINANTS OF ELEVATIONAL DISTRIBUTION IN HIMALAYAN BIRDS.

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HYPOXIA, HABITATS AND COMPETITION: DRIVERS OF DISTRIBUTION IN INDIAN MONTANE BIRDS.

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ABSTRACT

An important aspect of montane bird ecology is that most species have limited elevational distribution. We have a very poor understanding however, of the mechanisms that drive this limited elevational distribution in birds. In my dissertation, I investigated hypobaric hypoxia, interspecific competition and habitat loss as drivers of distribution in montane birds in the Himalayas. The first chapter is an introduction to the topic of elevational distribution in birds and the motivation behind the following chapters. In the second chapter we show that annual elevational movement patterns of Himalayan birds predict their strategy to cope with hypobaric hypoxia. We demonstrate that while elevational migrants increase hemoglobin concentration by increasing the volume of erythrocytes in their blood, sedentary high-elevation residents increase hemoglobin concentration by increasing mean cellular hemoglobin concentration in a comparative study of 15 passerine species. In the third chapter, I used song playback and feeder experiments to test the role of interspecific competition in driving the elevational distribution of green-backed and cinereous tits in the western Himalayas. I show that although green-backed tits are behaviorally dominant over

cinereous tits, cinereous tits live at high elevations by occupying habitats vacated by green-backed tits. In the fourth chapter, I used the results from the behavioral experiments to predict the spatial distribution of cinereous and green-backed tits in a landscape. Using occupancy modeling, we show that green-backed tits are dependent on forests for breeding sites at their elevational lower distributional limit and might be adversely affected by habitat loss. Cinereous tits, a common low elevation species on the other hand, occupy human-modified habitats and are likely expanding their elevational range upslope due to anthropogenic habitat modifications. In the fifth chapter, we undertook the first population ecology study on the green-backed tit in the Himalayas. Using a genetic blind test, we show that green-backed tit males have broader breast stripes than females. We also show that males and females have different beak shapes in winter but there is no significant difference in beak shape in summer due to male beaks becoming shorter.

BIOGRAPHICAL SKETCH

Sahas Barve was born in suburban Mumbai, India. Although surrounded by a concrete jungle, Sahas was brought up with stories of hunters and their adventures and the exploits of explorers in the wild outdoors. Sahas reveled in the stories of Jim Corbett, Kenneth Anderson and Teddy Roosevelt as told to him by his dad Sharad Barve. He asked his naturalist uncle Hari Divekar so many questions about the animals he had seen that Hari thought twice before visiting Sahas's place when he was a kid. Sahas was lucky to be the first generation in India to have cable TV while they were still in elementary school. Throughout middle school, Sahas watched and often re-watched episodes of natural history documentaries on the Discovery Channel. National Geographic TV and Animal Planet were yet to exist, at least in India. In eight grade Sahas attended a nature education camp organized by the Bombay Natural History Society and Nature Trails. Here he saw his first eagle, held his first snake and smelled his first wildflower.

In high school Sahas was busy learning Indian classical music, schoolwork, theatre and participating on the high school quiz team but always reading natural history magazines. In the summers, he would walk around Hari Divekar's properties in the countryside armed with his first pair of binoculars that Shubhada Marathe got him. In the summer after high school, armed with a new and better set of binoculars that Prachi Damle got him, he got a job as a naturalist taking high school students on a long hike everyday for two months. Sahas loved leading walks to show people the rich biodiversity that surrounds them. He went on to work for more than ten companies through college leading walks and organizing nature education camps.

Sahas really started learning ecology and evolution in Dr. Vasudevan's community ecology and Dr. Jhala's population biology class at the Wildlife Institute of India (WII) where he did a Masters degree. Sahas was elated to be living in a

campus far away from cities with Khaleej pheasants, leopards, monitor lizards and lot more wildlife running around the campus. He went on to do a dissertation on the differences in bird communities in young and old teak plantations and compared them to natural forests in the Western Ghats of India. Sahas also met his future wife Jennie Miller at WII. The Masters course has thus had quite the influence on Sahas' life.

Through three, field tech jobs in far-flung wild corners of India over two years post Masters, Sahas applied to grad schools in the US to continue studying birds. Sahas had some serious luck on his side as he got admitted to the PhD program at the Department of Ecology and Evolutionary Biology at Cornell. Sahas met André, his advisor in person only when he came to Ithaca to join the program. Thankfully they get along really well. The EEB Dept at Cornell is a great community and Sahas loved it here. Over the last five and half years Sahas has done a project in the Himalayas, published 9 papers/notes, gained invaluable field experience, made significant research connections and some strong friendships. Sahas plans to start a post-doctoral position in Eric Walter's Lab at Old Dominion University where he will study Acorn Woodpeckers in California.

[To Swati Barve and Sharad Barve]

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development. His support, advice and efforts and dedication towards graduate students at the Lab facilitated my ability to execute a field project in a far-flung corner of the world. His lab-meetings and discussion seminars are a constant source of friendly inspiration. He also gave me my first opportunity to review a paper and to teach a class completely independently. Harry Greene has been a great academic mentor. His ability to link natural history and basic biology with advanced evolutionary ecology is inspirational. I hope I continue to spread his word of understanding natural history of animals and their habitats and question our role as humans on Earth. Zac Cheviron "held my hand" through the physiological aspect of my thesis. He was patient and always made sure I fully understood something before I put it down on paper. Dr. Vinod Mathur is a great scientist and a great facilitator of science. I am one of the fortunate many who have been able to science under his supervision. Other academic mentors at Cornell include, in no particular order, Monica Geber, Keila Dhondt, Amanda Rodewald (thanks to you and your lab for some stimulating discussions), Walt Koenig, Mike Webster, David Winkler, Jeremy Searle, Kelly Zammudio, Stephen Emlen, Ken Rosenberg and Viviana Ruiz. It was absolutely wonderful teaching with John Fitzpatrick, Irby Lovette, Alex Flecker, Brian Lazarro, Abby Drake, Elizabeth Balko and Jeremy Searle. Frank La Sorte, Greg Budney, Martha Fischer, John Bowman, Matt Meddler, Jay McGowan were wonderful to work with at the Lab. Scott Robinson, Trevor Price, Farah Ishtiaq, V.V. Robin, and Christopher Witt all played significant roles in the development of my thesis. I am ever thankful to Janeen Orr and Carol Damm for being super efficient and caring administrators. Thanks to Zhila Sadri, Cindy Marquis and Micky Zifchock for bearing with all my small sum Indian receipts and being so nice about it. I am sure my accounts were a pain to deal with.

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them for making me feel welcome as I moved to and thrived in a new country. I have had some life changing discussions and just SO MUCH fun here. EEB at Cornell in the words of MG Weber is like a warm group hug. It's exactly that. Here are some great friends, again, in no particular order. Thanks to Suzi Claflin, Ben Johnson and Renee Petipas for just so many things, but also Holly Lutz, Sam Chamberlain and Hidetoshi Inamine for being champs and a great cohort to go down the PhD road with. Scott Taylor, Nick Mason, Nick Fletcher, Allison Tracy, Laura Porturas, David Toewes, Benjamin Freeman, Alexa Class-Freeman, Karissa Pena, Becca Brunner, Sue Pierre, Nancy Chen, Maya Lim, Leonardo Compagna, Kate Eisen, Cissy Ballen, Erin Larson, Bhavya Sridhar, Amanda Stephens, Natalie Hoffmeister, Josh La Pergola, Julie Miller, Julian Kapoor, Collin Edwards, Henry Kunerth, MG Weber, Gideon Bradburd, Rayna Bell, Chris Dalton, Gui Becker, Jacob Berv, Elliot Miller, Ruth Bennet, Sarah Wagner, Gerrardo Soto, Steven Rios, Gavin Leighton, and so many more for endless nerdery and fun. Adarsh Kowdle, Shreesha Shreenath, Tanay Gosawi, Shruti Gautam, Srinitya, Poornima Padmanabhan, Ajay Bhatt, Pooja Gudibanda, Parag Mahanti, Mukul Tikekar, Xuyao Mao, Aashish Sharma, Radhika Palkar, Reet and Swathi Rao for keeping the Indian in me alive and kicking.

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CHAPTER 1

INTRODUCTION

Charles Krebs defined ecology as the science that investigates the distribution and abundance of organisms (Krebs 1972). For most species (with the exception of invasive species), the latitudinal and longitudinal extent of a species' range represents limits set on its distribution by biological and environmental factors. Across its range, multiple mechanisms (e.g. interspecific competition, temperature) influence both the distribution and abundance of a species (Hutchinson 1957). Except for species endemic to small islands, it is often very difficult to study a species at the scale of its latitudinal or longitudinal distribution to understand the mechanisms influencing distribution and abundance along that axis. Montane species although often found across a large spatial extent are restricted to bands of elevation (Von Humboldt 1849). These elevational bands exhibit bounds in species distribution that span a small geographic distance and hence offer the opportunity to study the ecology of a species along an entire axis of its distribution relatively easily (Jankowski et al. 2013). Here I outline research conducted for my doctoral dissertation that explores mechanisms driving bird elevational distribution.

Several abiotic factors (e.g. partial pressure of oxygen, temperature) change predictably with gain in elevation. These in-turn influence biotic factors like habitats, predators and disease. Like latitudinal or longitudinal distribution, the elevational range of species is also driven by an interaction of biotic and abiotic mechanisms across contemporary and historic time scales. Nevertheless, the predictable change in crucial abiotic factors, a diversity of environmental regimes from tropical to temperate, fewer

sympatric species, a small geographic extent and increasingly significant local and global impacts of humans makes exploring the mechanisms driving elevational distribution an exciting and tractable pursuit critical for a better understanding of evolutionary ecology in general (Graham et al. 2014).

Studies on elevational distribution of birds range from several large scale comparative analyses of species distributions along elevational gradients (Graham et al. 2009) (Price et al. 2014, Diamond 1973) to very few small scale experimental investigations of hypotheses specific to a particular mechanism (Freeman 2016, Robinson and Terborgh 1995, Jankowski, Robinson, and Levey 2010). The most extensively investigated mechanism thus far has been interspecific competition. It has been considered as an important driver of vertebrate and plant elevational distribution (Randin et al. 2013, Hairston 1951, Pasch et al. 2013) for several decades. Interspecific competition has been studied especially well in birds starting from Jared Diamond's (1973) and Terborgh and Weske's (1975) influential papers on Andean and New Guinean birds. A slew of papers have since investigated and demonstrated the importance of competition in tropical mountain systems. Interspecific competition has however not been studied thoroughly on temperate mountains and studies suggest that temperature plays a bigger role in driving bird ranges than any biotic factor (Tingley et al. 2009).

The Himalayas, the highest mountains in the world, span a large latitudinal, longitudinal and elevational gradient. They are a world biodiversity hotspot (Myers et al. 2000) and are especially rich in avian biodiversity (Price et al. 2014). They show a strong a latitudinal diversity gradient from the seasonal and temperate northwest to the tropical southeast. Areas above 1500m in the northwest Himalayas are highly seasonal with cold

winters and mild summers. The high species diversity despite the temperate climate makes the western Himalayas a powerful system to test the general importance of several mechanisms driving the elevational distribution of species that have so far been tested only in tropical systems. Set largely within the most populous countries in the world, the Himalayas, and especially the western Himalayas, also face significant anthropogenic pressures largely through rampant land-conversions from forest to agriculture or pasture (Elsen et al. 2016) and thus present the opportunity to study the effect of habitat loss on the ecology of montane birds. However, a dearth of baseline data for most taxa is a serious challenge for research on species elevational distribution.

The seasonality, the spatial extent and the geographic location of the Himalayas has given rise to three major phenologies of spatial use among Himalayan birds; 1) Year-round residents, species that live at the same elevation all year round. There are resident species at all elevations; 2) Elevational migrants, species that breed at high elevations usually 2000m and above and winter at low elevations (below 2000m) and 3) Latitudinal migrants, species that breed in the Himalayas but winter throughout the Indian sub-continent from the Himalayan foothills all the way to Sri Lanka. These three groups of species and their elevational movements make avian diversity and richness along the elevational gradient dynamic, changing across seasons. There is little information about species elevational distribution throughout the year. In our field site in the Kedarnath Himalayas of Uttarakhand, India, we conducted surveys throughout the nearly 3000m elevational gradients to produce the first detailed dataset of species elevational ranges in winter and summer. This dataset Dixit, Joshi, and Barve (2016) shows three interesting patterns which are important to understand the elevational distribution of west Himalayan

birds; 1) Species richness at elevations above 2000m change drastically from winter to summer. High elevations have very few resident species that over-winter 2) Low elevations have similar species richness across seasons (Figure 1.1) and 3) There is very high turnover in species at all elevations but especially so at high elevations (Figure 1.2). High elevation habitats host 3-4 times as many species as winter. Naturally, these are elevational and latitudinal migrants using high-elevation habitats for breeding (Figure 1.2)

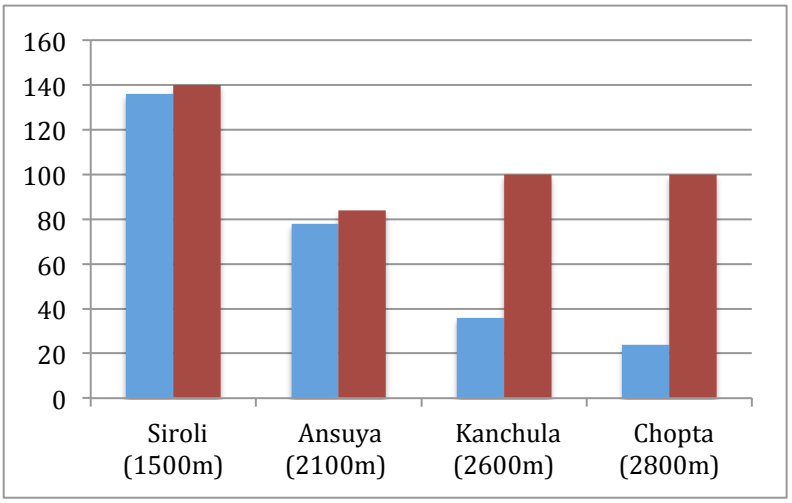

Figure 1.1: Species richness in winter (blue) and summer (red) at four elevations in the Kedarnath Himalayas. Figure from (Dixit et. al 2016)

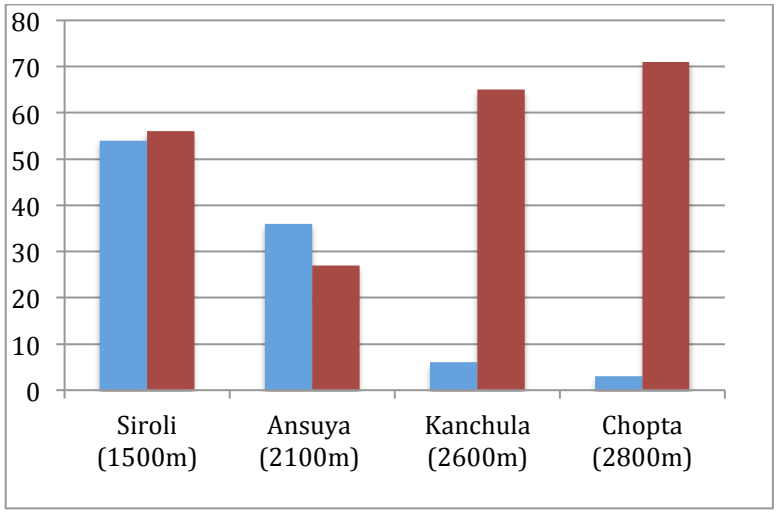

Figure 1.2: Turnover in species richness in winter (blue) and summer (red) at four locations along the elevational gradient in Kedarnath Himalayas. Figure from Dixit et al. 2016.

This detailed description of the avifauna of the field site played a crucial role in studying the ecological drivers of biodiversity distribution in an understudied landscape for this dissertation. Through this dissertation, I investigate the importance of physiology, competition and habitat in driving avian elevational ranges. In the first chapter I discuss results from a comparative physiology project that will surely inform future research on hypoxia physiology in the Himalayas and in birds in general.

The physiology of montane birds is likely most significantly affected by two abiotic factors that change predictably with elevation. Temperature and hypobaric hypoxia, both decrease with increasing elevation and can be significant constraints at high elevations (Jankowski et al. 2013). In fact, several studies have concluded that physiological tolerance often sets the upper limits on species distribution (Root 1988, Cadena et al. 2012, Londoño et al. 2015). Physiological tolerance is influenced by the ecology and evolutionary history of species (Price et al. 2011) and hence might affect species in a community differently. Since birds have a remarkable respiratory system

(Monge and Leon-Velarde 1991) most multi-species studies thus far have focused on the effect of temperature on the distribution of birds. However, both experimental (Londoño et al. 2015, Freeman 2015b) and observational studies (Freeman and Freeman 2014) have been unable to conclusively show that temperature is potentially limiting bird distribution in the tropics.

Although there is scant evidence to show that hypoxia limits species distribution, there is ample evidence to demonstrate that several high-elevation populations and species show physiological adaptations to cope with hypoxia (Storz, Scott, and Cheviron 2010). Sustained life in hypoxic conditions has repeatedly led to modifications in the physiology of high-elevation species. Most of the research on hypoxia physiology in the wild comes from a handful of high-elevation specialist species but Projecto-Garcia et al. (2013) show that substitutions in hemoglobin genes are seen in hummingbird species found as low as 2000m.

A first step towards a general understanding of how birds cope with hypoxia is to find common patterns in physiological responses to hypoxia in montane birds. Since physiological tolerance and responses are influenced by the ecology of the species, comparing species with similar life-history traits provides predictions that can be tested across a large number of species. In the first chapter, we tested whether elevational movement patterns in Himalayan birds predicted their strategy to vary blood hemoglobin (an important photo-pigment for oxygen transport) concentration. First we demonstrate that within species hemoglobin concentration increases with increase in elevation. We show that elevational migrants that breed at high elevations but winter at low elevations increase their hemoglobin concentration in response to hypoxia by increasing the volume

of erythrocytes in their blood (hematocrit). On the other hand, high elevation residents that are exposed to the same levels of hypoxia year round increase their hemoglobin concentration through increasing their mean cellular hemoglobin concentration. This is the first comparative physiological study to show that differences in elevational movement patterns drive fundamental differences in the way species regulate hemoglobin concentration. Physiological studies like these can be used in conjunction with studies on interspecific competition to understand the interaction between these two important mechanisms.

Most studies that have explored interspecific competition as a mechanism driving elevational zonation in birds have used current distributions of closely related elevationaly replacing species as evidence for the 'ghost of competition past' (Connell 1980)(Freeman 2015a). Despite being an active and current biotic interaction (Dhondt 2012), few studies quantify ongoing competitive interactions between elevationaly replacing species in areas of range overlap (Jankowski, Robinson, and Levey 2010). There is a need for experimental studies on elevationaly replacing species pairs despite their small spatial scale as ecological and evolutionary mechanisms interacting at local scales can have large-scale implications.

Research that has used behavioral experiments to study competition largely involves hetero-specific song-playback experiments. These simulated territory invasions have revealed two important patterns; 1) Response to song-playbacks is often asymmetric where one species shows a significantly stronger response than the other and 2) the strength of this response for both species is highest at the range boundary suggesting that it might be a learned response. Although observing heterospecific aggression in nature is

difficult, the intensity of response to song playbacks suggests that aggression plays an important role in maintaining territory and range boundaries between species (Peiman and Robinson 2010). The biggest criticism of the studies of competition using song playbacks is that it is difficult to prove that the lack of response of one species is a signal of its subordination. However, the lack of long-term studies and information on limiting resources has led to the wide use of song playbacks in the tropics. Song-playbacks have also been successful in the tropics since species have long breeding seasons and are often territorial year-round.

Temperate habitats like the western Himalayas are different from the tropics in many ways. They have large seasonal fluctuations in temperature and hence primary productivity across seasons. This seasonality leads to shorter breeding seasons, resources being scattered over larger spatial scales in winter and thus birds being less territorial outside the breeding season. Consequently, the resources that are limiting in the summer are not necessarily limiting in winter (Dhondt 2012). Temperate mountains thus present the exceptional opportunity to study competition among closely related elevationaly replacing resident species across seasons and resources regimes. Studying interactions between resident species is important since it can reveal the general importance of interspecific competition in driving elevational ranges in temperate habitats. Studying interactions over distinct resources also confirms the direction and intensity of dominance among species through competitive interactions over distinct resources.

Along natural slopes, cinereous tits and green-backed tits co-occur in a narrow zone of overlap at the ecotone of subtropical pines and oak forests in the western Himalayas (~1300m). However cinereous tits are found in human settlements and nearby

human-altered habitats up to 1600m; 300m above their natural distribution (Dixit, Joshi, and Barve 2016). This pattern of distribution can arise due to cinereous tits displacing green-backed tits from human-altered habitats or green-backed tits choosing to breed away from human habitats. In the second chapter of this dissertation, we carried out systematic behavioral experiments to understand the nature and direction of competitive interactions between the cinereous tit (*Parus cinereous*) and the green-backed tit (*Parus monticolus*) in the western Himalayas. Then in the third chapter, we tested whether the fin scale spatial distribution of these two species is predicted by the outcomes of their competitive interactions.

Green-backed tits were behaviorally dominant over cinereous tits in both winter and summer. Green-backed tits aggressively displaced cinereous tits at concentrated food sources (feeders) in the winter and showed a strong aggressive response to cinereous tit song (reciprocal song-playbacks) in the breeding season. Through distinct behavioral experiments across seasons we can uniquely demonstrate that the heterospecific aggression by green-backed tits towards cinereous tits seen in the breeding season is manifested in competitive interactions over food in the non-breeding season. Thus the direction and intensity of hetero-specific aggression is maintained throughout the year in this species pair.

The cinereous tit-green-backed tit system offered the opportunity to test whether known patterns of heterospecific aggression drive the fine scale spatial distribution of these two species in the sympatric zone. Through year-round point-count surveys across three valleys (1500m -1580m), we investigated two questions about spatial distribution of elevationally replacing species in the range-overlap zone in an occupancy framework. 1)

What habitat parameters best predict the occupancy of these two species in the overlap zone and 2) Is the occupancy of one species conditional on the site being occupied by the other species? We show that green-backed tits are restricted to forests in the breeding season and to habitats with high tree density in the winter. Cinereous tits on the other hand are highly associated with human settlements throughout the year. However the occupancy of neither species is conditional on the site occupancy of the other species. Our results demonstrate a strong role of habitats driving the distribution of the two species in the sympatric zone. Green-backed tits despite being behaviorally dominant are restricted to natural habitats away from human settlements. Cinereous tits have thus successfully colonized human-altered habitats above their natural distribution by occupying habitats that are sub-optimal for green-backed tits. Local-scale anthropogenic habitat changes are likely leading to changes in Himalayan bird elevational distribution.

Our occupancy studies indicate that several other species of human commensal birds are found at elevations above their natural distribution with a majority of detections occurring only close to human habitations. This suggests a general change in the Himalayan bird community driven by human habitat modifications where generalist commensal species are occupying areas formerly occupied by montane species. It is thus increasingly crucial to study the effect of habitat change on the elevational distribution of birds not only in the Himalayas but all the high mountains of the world.

The dearth of basic population biology on most Himalayan species, barring a few groups (Ghosh-Harihar and Price 2014), currently hampers the advancement of avian evolutionary ecology in the Himalayas. We took a step in this direction by doing the first population ecology study of green-backed tits in the Himalayas. Although breast stripe

dimorphism is known and widely studied in the great tit (*Parus major*) (Figuerola and Senar 2000), differences in breast stipe width has never been quantified in the green-backed tit. We demonstrate that breast stripe width is correlated with male condition in winter and is a reliable measure to sex this species in the wild. We also describe the sexual dimorphism in eco-morphological variables in the study population. Sexual dimorphism in beak morphology changed from winter to summer through male beaks shortening 4.5 % in summer with little difference in female beak measurements. These differences in beak morphology have been documented in great tits in Europe (Gosler 1987) suggesting that temperate Himalayan forests drive similar differences in foraging between green-backed tit males and females as temperate European forests. Several Himalayan birds have closely-related, well-studied species in the Palearctic. Such species might be good candidates for the study of population ecology in this under-studied fauna.

This dissertation has facilitated research on the elevational distribution of Himalayan birds in the following way. 1) We have generated a detailed baseline dataset of year-round elevational distribution of Himalayan birds for our study area. We hope this is dataset is used for comparison with other locations of the Himalayas and the same region in the future to study the effect of anthropogenic impacts on bird distribution. 2) We carried out the first comparative physiological study of one strategy used by Himalayan birds to cope with hypoxia. 3) We explored the role of interspecific competition and habitats in driving the distribution of two elevationaly replacing species through meticulous behavioral experiments and occupancy surveys. We concluded that habitat is more important than competition in driving the distribution of these two species. 4) Finally we carried out the first population study of green-backed tits in the

Himalayas. We explored plumage and beak dimorphism to show that green-backed tits in the Himalayas show ecological similarities to great tits in Europe.

Findings from this dissertation indicate two important directions for future research. The first understands how biotic and abiotic mechanisms interactively shape the elevational distribution of species. With the direction of dominance and aggression known between green-backed tits and cinereous tits, this species pair can be used in aviary experiments to understand how hypoxia and temperature affect the competitive ability of species. These experiments can also shed light on the ability of these species to acclimate to higher or lower elevations and their stress response when exposed to conditions outside their natural distributional range. Emerging technology like thermal imaging can also be used to measure differences in heat loss at night as a mechanism restricting species to lower elevations.

The other important avenue of research involves understanding the up-slope range expansion of low elevation species through association with humans. The effect of anthropogenic habitat alterations on species distribution is widely studied around the world, however it is not considered an important driver of species elevational distribution. Increasing human populations in montane areas around the world suggest that habitat loss might contract the ranges of several mid- elevation birds in the future. Hence, understanding current habitat use and movement ecology of low elevation species at their upper distributional limit is not only important for the conservation of biodiversity in this hotspot but also generally important to understand how species ranges expand through association with humans.

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CHAPTER 2

Life history characteristics influence physiological strategies to cope with hypoxia in Himalayan birds.

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Abstract

Hypobaric hypoxia at high elevation represents an important physiological stressor for montane organisms, but optimal physiological strategies to cope with hypoxia may vary among species with different life histories. Montane birds exhibit a range of migration patterns; elevational migrants breed at high elevations but winter at low elevations or migrate further south, while high-elevation residents inhabit the same elevation throughout the year. Optimal physiological strategies to cope with hypoxia might therefore differ between species that exhibit these two migratory patterns because they differ in the amount time spent at high elevation. We examined physiological parameters associated with blood-oxygen transport [haemoglobin concentration and haematocrit (the proportion of red blood cells in blood)] in 9 species of elevational migrants and 6 species high-elevation residents that were sampled along a 2200m (1000m-3200m) elevational gradient. Haemoglobin concentration increased with elevation within species regardless of migratory strategy, but it was only significantly correlated with haematocrit in elevational migrants. Surprisingly, haemoglobin concentration was not correlated with haematocrit in high-elevation residents, and these species exhibited higher mean cellular haemoglobin concentration than elevational migrants. Thus, alternative physiological strategies to regulate haemoglobin concentration and blood O_2 carrying capacity appear to differ among birds with different annual elevational movement patterns.

Key-words

Haemoglobin concentration, haematocrit, elevational migration, birds, comparative physiology, Himalayas

1. Introduction

A primary goal of comparative physiology is to determine whether ecological similarities among species might explain similarities in their adaptive responses to common selective pressures (Monge and Leon-Velarde 1991). Matching migratory strategies, for instance, can influence the temporal and spatial variation in environmental stressors individuals of a species experience over their lifetime. As a result, species that share key life history traits may exhibit analogous physiological strategies to cope with the same environmental stressor (Badyaev and Ghalambor 2001). Species that are distributed along elevational gradients are powerful systems to investigate the influence of life history variation on physiological adaptation. Key abiotic stressors such as temperature and biotic stressors such as interspecific competition (Price 1991) change with elevation. Certain abiotic stressors intensify predictably with elevation (e.g. decrease in temperature with increase in elevation), and dramatic changes in these stressors occur over relatively small spatial scales. Perhaps most notable is the decrease in the partial pressure of oxygen (hypobaric hypoxia) at high elevations (Storz 2010). Hypobaric hypoxia is constant and predictable at a given elevation around the world throughout the year and is not affected by latitude or season making it a unique stressor for high elevation organisms globally. Organisms cope with hypoxia using diverse physiological and behavioural strategies (Monge and Leon-Velarde 1991), but systematic analyses of the influence of life history characteristics on the utilization of these alternative strategies are rare.

Studies of physiological responses to hypoxia in laboratory animals (e.g. guinea pigs and rats) (Blake and Banchero 1985, Costa, Mendez, and Boveris 1997), humans

(Moore, Niermeyer, and Zamudio 1998, Beall et al. 2010), and free living wild species(Cheviron and Brumfield 2012, Sakai et al. 2003) have revealed a diverse suite of physiological modifications that species can use to counter hypoxia. Among the most common are increases in pulmonary O_2 transport (Crowell and Smith 1967) and modification of blood O_2 carrying capacity (Schuler et al. 2010). An important determinant of blood O_2 carrying capacity is haemoglobin concentration (henceforth [Hb]) (Velguth, Payton, and Hoover 2010, Tufts et al. 2013). Erythropoietic responses to hypobaric hypoxia can increase blood O_2 carrying capacity by increasing [Hb] via the proliferation of red blood cells, which leads to an increase in haematocrit (henceforth Hct), the proportion of whole blood volume that is occupied by erythrocytes. This erythropoietic response to hypoxia is well documented in birds (Crowell, Ford and Lewis 1959) and other vertebrates that routinely migrate between highland and lowland environments (Villafuerte, Cardenas, and Monge 2004). Although this response allows of fine-tuning of blood O_2 carrying capacity as a potentially effective short-term solution to hypoxic challenge, the adaptive value of chronically elevated Hct in high-elevation organisms is questionable.

Although increasing [Hb] through increases in Hct can increase blood O_2 carrying capacity, elevated Hct also increases blood viscosity, and excessive blood viscosity can increase cardiac load and hampers effective blood circulation, both of which may ultimately decrease convective oxygen delivery. Given these considerations, several studies have suggested that Hct and [Hb] values that optimize aerobic output in humans are very close to those typically observed at sea-level (Crowell and Smith 1967, Schuler et al. 2010, Tufts et al. 2013, Crowell, Ford, and Lewis 1959). Not only does chronically

elevated Hct potentially reduce aerobic output, it is associated with pathophysiological conditions in humans (e.g. chronic mountain disease), and correspondingly, many high-elevation specialist species have evolved blunted erythropoietic responses to hypoxia (Villafuerte, Cardenas, and Monge 2004) (normal Hct at high elevations). Thus, the optimal erythropoietic response to hypoxia may vary according to the amount of time an organism spends under hypoxic conditions.

Birds in the Himalayas exhibit two distinct phenologies of elevational distribution. Elevational migrants occur at low elevation for most of the year (8-9 months), but move to higher elevations (2000m - 4000m or even higher) during the 3-4 month summer to breed (Dixit, Joshi, and Barve 2016). These two elevational movement strategies are widely known in birds and have been hypothesized to arise due to limits on cold tolerance in winter (Carey and Morton 1976), food availability (Boyle, Sandercock, and Martin 2015) and predation (Boyle 2008). These differences result in elevational migrants enduring significant hypoxic stress only for a short period of the year compared to high-elevational residents, which occur at high elevation throughout the year. Given their contrasting elevational movement patterns, elevational migrants and resident birds present a good system with which to understand if elevational movement patterns explain the strategies to cope with environmental hypoxia through erythropoietic regulation of blood O_2 carrying capacity.

In this study, we used a survey of 15 avian species that differ in migratory strategies to demonstrate that erythropoietic responses to increasing elevation can be explained by interspecific differences in annual elevational movement patterns. We predicted elevational migrants would show a seasonal increase in [Hb] with a correlated

increase in Hct during the breeding season because the ability to finely tune blood O_2 carrying capacity may outweigh the maladaptive, but transient increases in blood viscosity. High-elevation residents on the other hand, experience chronic hypoxia, and may thus exhibit alternative strategies for coping with hypobaric hypoxia, which may include a blunted erythropoietic responses to increasing elevation as has been documented in other high-elevation specialists. Using this comparative approach, we demonstrate that differences in species annual elevational movement patterns (elevational migrants and high-elevation residents) can explain differences in their physiological responses to abiotic stressors. To our knowledge, this is the first study to link interspecific variation in migratory strategies to variation in erythropoietic responses to hypoxia.

2. Materials and Methods:

(a) Field methods

We obtained blood samples from 15 species of passerine birds (Figure 2.1) that we caught opportunistically using mist-nets at 7 elevations, 1000m, 1500m, 2100m, 2650m, 2800m, 3000m and 3200m in the Amrut Ganga Valley of Kedarnath Wildlife Division, Uttarakhand, India, These species were selected based on their abundance and diversity in elevational distribution. We restricted our analyses to passerine species to minimize the effect of evolutionary distance on differences in physiology. We sampled birds in two seasons, summer/breeding season (March 15-June 30) and winter/non-breeding season (January 1- March 15) in 2014 and 2015. We used March 15th across both years of sampling as the date for the onset of the breeding season (summer) as resident birds were

commonly seen singing about a week before this date (Dixit, Joshi, and Barve 2016). To ensure that we did not sample elevational migrants that had just arrived on their breeding grounds and not acclimatized to the hypoxia, we sampled at and above 2600m only in late April and May. Since our study sites spanned an elevational gradient larger than the distribution of most species, at each sampling location, only a subset of the species sampled was present (Figure 2.1). For each blood sample, we measured whole blood [Hb] with a Hemocue 201+ analyser using the manufacturer's protocol (HemoCue AB, Ängelholm, Sweden) (N (breeding season) = 356). Whenever sufficient sample was available, we also measured Hct for the same samples, using a Zipocrit Portable Centrifuge (LW Scientific Inc., Lawrenceville, GA, USA) with a spin time of five minutes (N (breeding season) = 178, N (non breeding season)=152)). We calculated the Mean Cellular Haemoglobin Concentration (henceforth MCHC) using the formula MCHC= ([Hb] X 100/Hct) (N (breeding season) = 178, N (non breeding season)=152)(Chappell, Bachman, and Odell 1995). We checked for repeatability of our measures by taking two successive measurements from the same bird for [Hb] (n=21 birds) and Hct (n=23 birds), and calculating the intraclass correlation coefficient (ICC) using the package ICC in the R software package 3.2.1 (Wolak, Fairbairn, and Paulsen 2012). For both measures we had high ICC scores (Hb= 0.85, Hct=0.98) confirming our measurements were highly repeatable.

(b) Data analyses

Based on information from field surveys (Dixit, Joshi, and Barve 2016) and information from literature (Ali and Ripley 1983), we determined elevational distribution (in both

winter and summer) of each species and mass (g) (Supplementary info 1). Most birds in the Himalayas show some elevational movement especially during extreme weather events. We classified birds as elevational migrants (elevational migrant status=1) for species where almost all individuals migrate to lower elevations from the upper 75% of their breeding range. Sedentary residents (elevational migrants =0) were species that are consistently found in 75% of their breeding range in the winter in our study site.

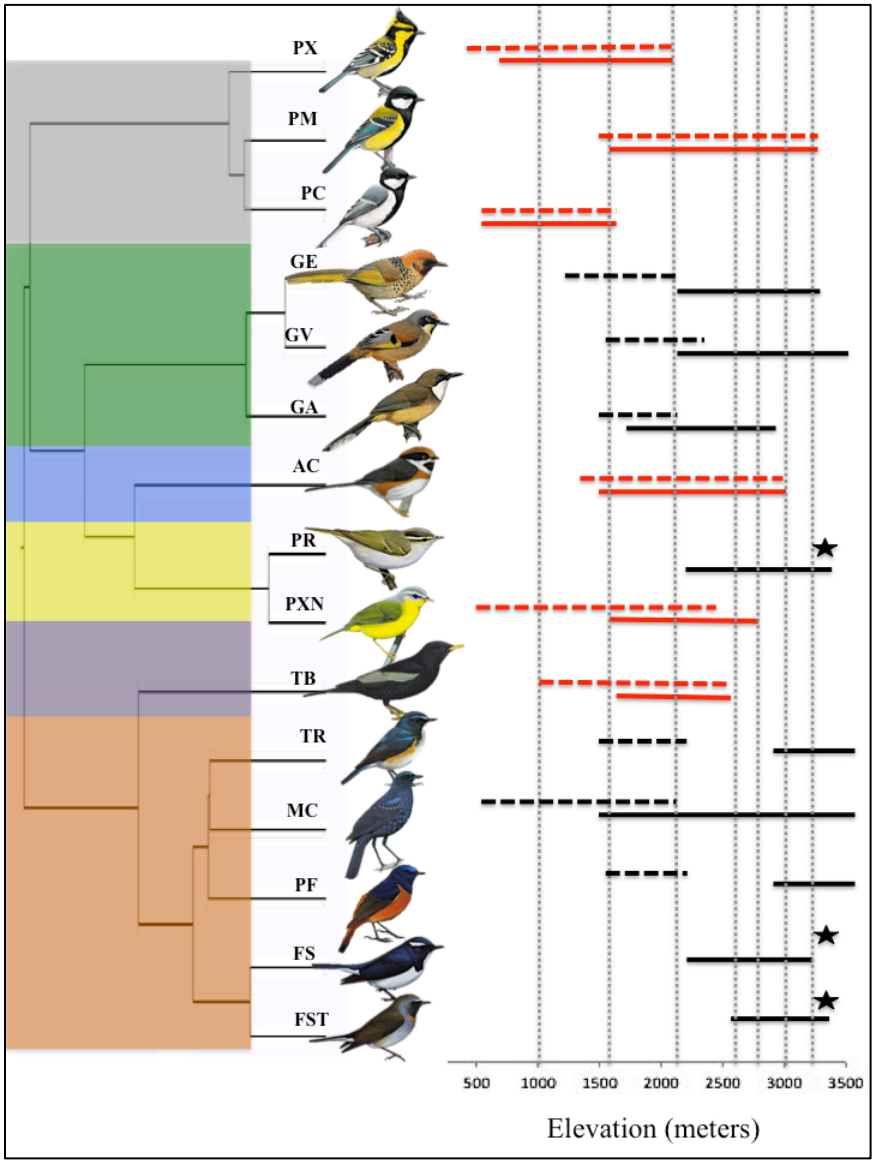

Figure 2.1: Evolutionary relationships among species used in the analysis with phylogeny re-created using a posterior set of pruned trees from Jetz et al. (2012). Elevational distributions are from Dixit et al (2016). Dotted vertical lines represent sampling locations. Horizontal solid lines represent species breeding elevational distribution and dashed lines represent species winter distribution (in meters) of resident (red) and elevational migrant (black) species. Stars indicate species with winter distribution outside the study area. Coloured boxes on the left margin denote taxonomic families used in the analysis; grey- Paridae, green- Timalidae, blue-Aegithalidae, yellow- Sylvidae, purple- Turdidae, buff- Muscicapidae. Species codes (from top) PX (*Parus xanthogenys*), PM (*Parus monticolus*), PC (*Parus cinereus*), GE (*Garrulax erythrocephalus*), GV (*Garrulax variegatum*), GA (*Garrulax albogularis*), AC (*Aegithalos concinnus*), PR (*Phylloscopus reguloides*), PXN (*Phylloscopus xanthoschistos*), TB (*Turdus boulboul*), TR (*Tarsiger rufilatus*), MC (*Myophonus caeruleus*), PF (*Phoenicurus frontalis*), FS (*Ficedula superciliaris*), FST (*Ficedula strophiata*). Bird illustrations were reproduced from Handbook to the Birds of the World Alive (del Hoyo et al. 2014).

To understand the variation in the relationship between [Hb] and Hct among species that exhibit the two alternative elevational movement patterns, we quantified the strength of the relationship between [Hb] and Hct separately in elevational migrants using linear regression (9 species) and residents (6 species). To further understand the mode of increase in [Hb] in individuals showing elevated [Hb], we compared the MCHC in the upper quartile values of [Hb] between elevational migrants and residents. Finally, using data for 15 species in summer and 9 species in winter, we fit 10 linear mixed models and used AIC-based multi-model inference to identify well-supported statistical models that describe the relationships between [Hb] and biological parameters relevant to elevational distribution such as 1) Hct 2) elevational range 3) range upper limit (highest limit of distribution for the species in the landscape) 4) range position calculated as [(elevation where bird was caught - lower elevational limit of distribution)/elevational range of species] 5) elevational migrant (EM) status (1 = Elevational migrants, 0 = Resident species) and 6) Mass (g) as fixed effects. In these models, we controlled for phylogenetic effects by including a phylogenetic correlation matrix as a random effect using the statistical package ape (Paradis, Claude, and Strimmer 2004). The phylogenetic correlation matrix was derived from a phylogeny constructed from information in Jetz et. al (2012). Since, we include species from multiple taxonomic families in our analysis, the Jetz et al. (2012) phylogeny is likely a satisfactorily accurate representation of the evolutionary relationships and genetic distance between the species. The models were written based on predictably important ecological variables and their interactions relevant to the elevational distribution of a species, such that each model represented a specific hypothesis. Each model had the same structure ([Hb]~ Predictor 1+Predictor 2 +

Predictor 1*Predictor 2+ (1|Species)). The variables used in each model are given in table 1. For predictor variables that were highly correlated ($r > 0.6$) (Bates et al. 2014), we retained those variables that were ecologically relevant to hypoxia physiology, for example, we retained "upper limit" rather than the "lower limit" of the elevational distribution of a species. A complete description of models is given in Table 2.1. For all models, we included "Species" as a random intercept effect. We fit the linear mixed models using the lmekin function of the coxme package (Therneau 2012) and model selection was done using the MuMIn package (Barton 2011). All analyses were done in the software package R 3.2.1 (R Development Core Team 2015).

Results

There was a significant positive relationship between [Hb] and elevation in both elevational migrants (R^2=0.24, $p<0.001$) and high elevation residents (R^2=0.16, $p<0.001$) (Figure 2.2a, 2.2b). However, we found that the strength of the association between [Hb] and Hct is a function of the migratory status of a species. Although elevational migrants exhibit a strong positive relationship between [Hb] and Hct (r^2=0.42, $P<0.001$), there was no significant relationship between the two variables in resident species (r^2=0.003, $P>0.35$), suggesting an uncoupling of [Hb] and Hct in resident birds (Figure 2.2c and 2.2d). Moreover, MCHC was significantly higher in sedentary residents than elevational migrants ($t= 3.2076$, $df=45$, $P<0.01$), suggesting that erythrocytes of high elevation residents contain more haemoglobin than those of elevational migrants. In the breeding season linear mixed model analysis, controlling for phylogeny, the best-fit model (Model

3, Hb~Hct+EM_Hct*EM+(1|Species)) describes variation in [Hb] as a product of Hct, elevational migrant (EM) status (1,0) and an interaction between Hct and EM (Table 2.1).

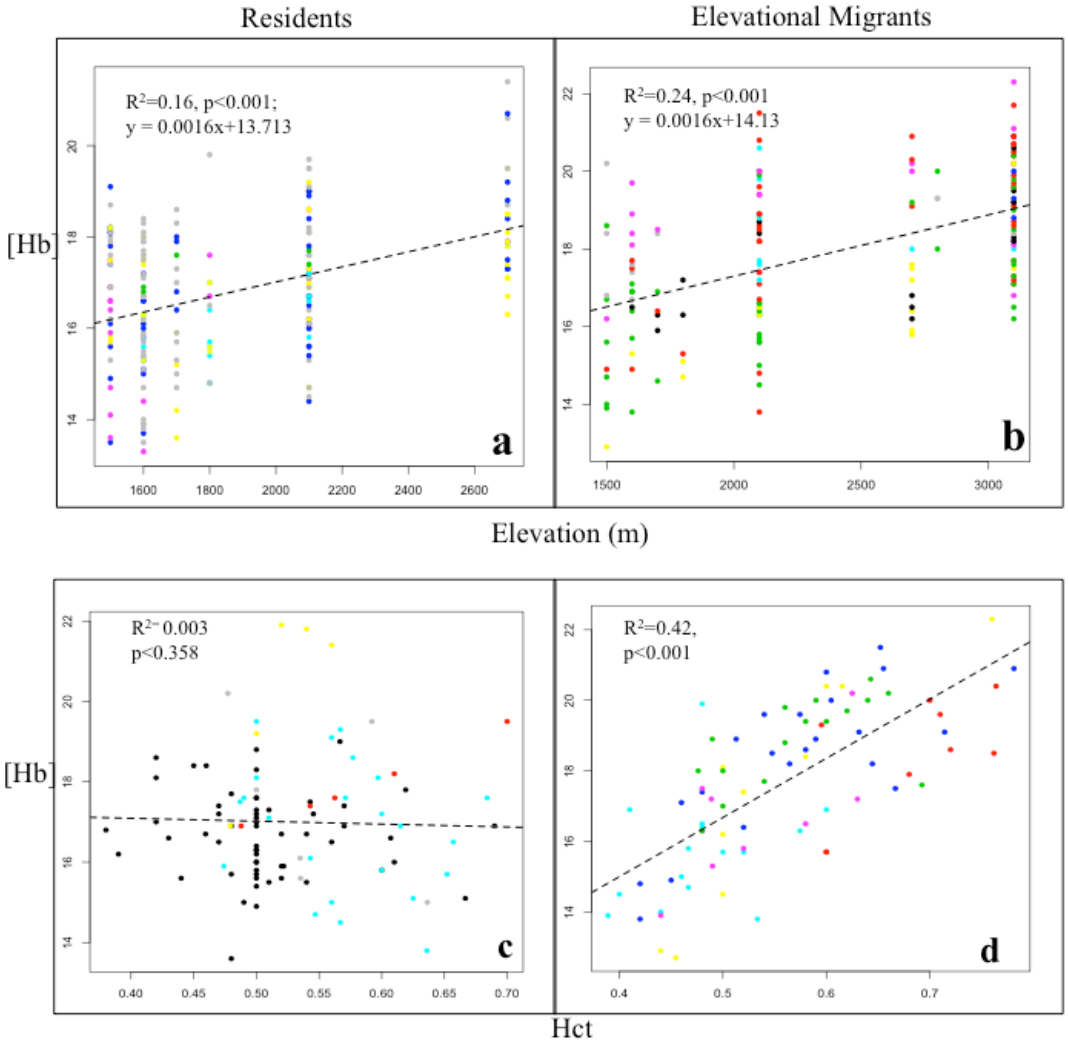

Figure 2.2: Haemoglobin concentration [Hb] (g/dl) in residents and elevational migrants increases with elevation (a, b). [Hb] is not correlated with haematocrit (Hct) in residents (2c) but is highly correlated in elevational migrants (2d).

Model #	Hct	EM	Mass	Range	Elevation	Upper limit	Range position	df	ΔAICc
3	X	X						6	0
4	X					X		6	12.33
6	X			X				6	14.39
2	X				X			6	17.73
9	X						X	6	22.2
1	X	X	X	X	X	X	X	11	27.66
5					X	X		6	33.84
10					X		X	6	35.81
7				X		X		6	37.22
8			X	X				6	37.79

Table 2.1: Results of model selection of linear mixed models to investigate specific hypotheses predicting Hb concentration. (Hct: haematocrit; EM: Elevational Migrant (1,0); Mass: Mass of the individual (in grams); Range: elevational range in meters; Range Position: Proportion of range where sampled; Elevation: Elevation at which sampled; Upper limit: Highest limit of elevational distribution). X indicates predictors used as fixed effects in the model. Species was used as a random intercept effect in all models. All models include an interaction between the two variables in the model. Models are arranged more models showing highest fit to models showing lowest fit.

This model was weighted appreciably higher than the any other model (Table 2.1). Hct and EM have weak effects but the interaction between Hct and EM has statistically significant positive effect on the variation of [Hb] (Figure 2.3). This result coupled with the differences in the strength of the relationship between [Hb] and Hct in migrants and residents suggests that residents exhibit a common and functionally different mechanism

for regulating [Hb] than migrant species (Figure 2.2). In the non-breeding season, the global model (including all fixed effects) is selected as the top model in the linear mixed model analysis.

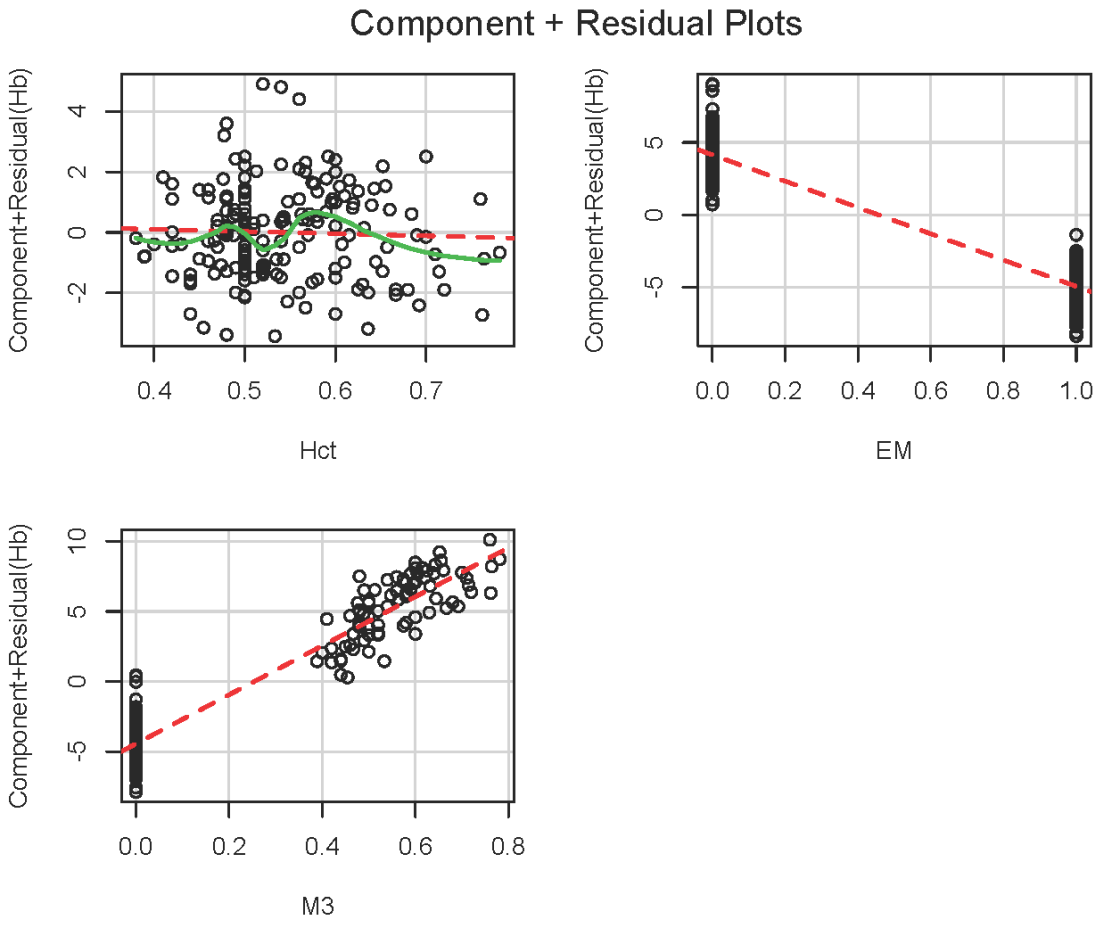

Figure 2.3: Partial residual plots for top model in linear mixed model analysis [Hb~Hct+EM+Hct*EM+(1|Species)]. M3 denotes the interaction factor between Hct and EM (Elevational Migration Status)

Discussion

Here, we show that although both high-elevation residents and elevational migrants modulate [Hb] to increase blood O_2 carrying capacity in the face of

environmental hypoxia (Figure 2.2a, 2.2b), they achieve elevated [Hb] at high elevation through different means. The strong correlation between [Hb] and Hct in elevational migrants suggests retention of an ancestral strategy where lowland taxa increase [Hb] through up regulation of erythrocyte production (Figure 2.2d)(Storz, Scott, and Cheviron 2010). The lack of such a correlation in high-elevation resident species (Figure 2.2c) suggests that species that consistently reside at high elevations regulate [Hb] independent of increases in Hct. Such a strategy would be particularly advantageous for highland residents as it would allow them to increase blood O_2 carrying capacity without the concomitant increases in blood viscosity incurred by excessive erythropoiesis (Beall 2002).

An increase in [Hb] can be brought about by several mechanisms other than increasing Hct (Ramirez, Folkow, and Blix 2007). Perhaps most important is to simply increase the [Hb] of individual erythrocytes (i.e. to increase the mean cellular haemoglobin concentration – MCHC). Consistent with this expectation, we found that MCHC was significantly higher in resident species than elevational migrants residing at high elevation ($t= 3.2076$, $df=45$, $P<0.01$). Together, our results suggest that high elevation residents likely increase [Hb] through an increase in MCHC while elevational migrants increase [Hb] through an increase in Hct. An increase in the MCHC may lead to an increase in erythrocyte size reducing oxygen diffusion to tissues (Canals et al. 2007)(Monge and Leon-Velarde 1991) suggesting that there is likely a threshold to increase in MCHC. Highland mammals are therefore known to have smaller erythrocytes to increase oxygen transport than low-elevation conspecifics (Hawkey et al. 1991,Tufts et

al. 2013). Due to logistical reasons we could not measure erythrocyte size in our study species.

In contrast to high-elevation residents, elevational migrants spend only a short period of time at high elevations every year. This life-history strategy may require the ability to finely tune blood O_2 carrying capacity throughout the year. A correlated increase in [Hb] and Hct is the most commonly documented acclimatization response to environmental hypoxia in birds and other vertebrates (Borras, Cabrera, and Senar 2010, Moore, Niermeyer, and Zamudio 1998, Carey and Morton 1976). Hct exhibits high degree of phenotypic flexibility in most vertebrates (Birchard 1997, Tufts et al. 2013). Thus, Hct mediated changes in blood O_2 carrying capacity may provide elevational migrants with the ability to finely-tune [Hb] throughout the year, and the increase in blood O_2 carrying capacity brought about by increases in Hct might offset the costs of increased blood viscosity for during the relatively short breeding seasons at high elevation.

Although migratory strategy was the principal predictor of variation in [Hb] in our linear mixed model analyses, the effect of migratory strategy was much more pronounced in the breeding season compared to the non-breeding season. The best fit model in the winter (non breeding season) was the global model that included all the fixed effect variables. This apparent discrepancy may be explained by the fact that all of the elevational migrants were sampled at low elevations (1000m-2600m) during periods of low physiological hypoxic stress and therefore exhibiting low [Hb] and Hct values.

An important additional element of blood O_2 carrying capacity is Hb-O_2 affinity. Due to difficult field logistics we could not freeze blood samples to carry out this

analysis. We therefore did not sequence the haemoglobin genes in these birds as functional data are essential to understand the phenotypic consequences of substitutions in haemoglobin genes (Natarajan et al. 2013). Future studies should therefore carry out functional assays of Hb-O_2 affinity and couple them with studies of sequence polymorphisms in haemoglobin genes (Projecto-Garcia et al. 2013, Storz et al. 2010) to better understand the mechanisms underlying the distinct strategies in [Hb] regulation between elevational migrants and high-elevation residents.

The extent to which hypobaric hypoxia might limit bird species distribution is not well understood (Jankowski et al. 2013). A first step towards a general understanding of how bird species distribution might be restricted by hypoxia is to study common patterns in physiological responses in wild birds. Understanding interspecific variation in hypoxia tolerance is increasingly critical in light of climate change since hypoxia is unaffected by increasing temperatures and several montane species are predicted to show an upslope range expansion. Our study demonstrates that elevational migration, a common life history strategy in montane birds, can explain inter-specific differences in physiological strategies to cope with hypoxia. Although several Himalayan passerine species occur at elevations higher than our highest sampling site, the elevational range we sampled harbours much higher avian bird diversity than areas above 4000m, both in the Himalayas and across the globe (Elsen and Tingley 2015). Furthermore, the upper elevations we did sample appear to be sufficient to induce hypoxic stress in birds as parallel genetic mutations to increase Hb-O_2 affinity have evolved at elevations as low as 2000m in Andean hummingbirds (Projecto-Garcia et al. 2013). In the Himalayas, up to 65% of the species that breed at the highest elevations are elevational migrants (Dixit,

Joshi, and Barve 2016, Somveille et al. 2013) which thus make up a significant part of the bird community at these elevations in the breeding season. Our findings show that elevational migrants exhibit an erythropoietic response to environmental hypoxia that is typical of lowland species. This ancestral, erythropoietic response and its associated costs may affect the rate of upslope migration even if thermal conditions are favourable (Storz, Scott, and Cheviron 2010). Thus, incorporating information on the specific physiological mechanisms that individual species employ to cope with hypoxia might be crucial for accurately estimating range shifts in high elevation montane birds.

To our knowledge, our study is the first to explore the erythropoietic response to hypoxia at the level of a bird community along an elevational gradient. This has revealed common strategies in the response to hypoxia strongly associated with the species' pattern of elevational movement. Several biotic and abiotic factors might simultaneously affect the elevational distribution of a species. For example, hypoxic stress might influence outcomes of biotic interactions such as interspecific competition and rates upslope range shifts in low elevation birds might be affected by the interaction between such biotic and abiotic stressors. Interactive effects of covarying stressors as constraints on species distributions will require increasing attention if projections of species distributions are to be accurate (Graham et al. 2014). Our findings demonstrate that strategies to cope with hypoxia are associated with life history traits and might be generalizable across species to yield predictions for other high mountain systems like the Andes where movement patterns of birds are more poorly understood and more difficult to study.

Ethics

Appropriate permissions were obtained from the Uttarakhand State (India) Forest Department for sampling of birds. Conventional mist-netting protocols were used during sampling.

Data Accessibility

Supplementary information is available as **Barve et al Suppl Material 1.pdf**

Competing interests

We declare we have no competing interests.

Author contributions

S.B. conceived and designed the study, acquired data, carried out the analysis and drafted the manuscript. A.A.D and Z.A.C. designed the study and developed the manuscript. V.B.M. designed the study, acquired data and developed the manuscript.

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CHAPTER 3

Competitively subordinate cinereous tits persist with dominant green-backed tits at high elevation by occupying human modified habitats in the Himalayas.

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Abstract

Elevational species replacement is a widely documented pattern in montane species distribution. Although interspecific competition has been shown to be important in setting species elevational limits in tropical habitats, its effect in species of temperate habitats is poorly studied. We tested whether interspecific competition for space in the breeding season and for food in the non-breeding season would drive the distribution of two resident titmice species in the Himalayas. High elevation green-backed tits (*Parus monticolus*) are behaviorally dominant over low elevation cinereous tits (*Parus cinereus*) in both song playback and feeder trials. Despite being subordinate, cinereous tits persist at high elevation in the sympatric areas by occupying human modified habitats. Our study suggests that the loss of natural habitats in the sympatric zone, not interspecific competition, might be limiting the distribution of the high-elevation green-backed tits and facilitating an upward range shift through human association in cinereous tits.

Key words: song playback, interspecific competition, elevational distribution

Introduction

The elevational distribution of a species is shaped by a combination of ecological and environmental factors (Graham et al. 2014). Instances where closely related species supplant each other along the elevational gradient, a global pattern known as elevational species replacement, offer powerful systems to study ecological interactions that drive the elevational distribution of species. Interspecific competition is considered a critical ecological mechanism restricting elevational distribution and maintaining elevational

range boundaries in several vertebrate taxa showing elevational species replacement (Hairston 1951, Diamond 1973, Pasch et al. 2013).

Heterospecific song playbacks have been widely used in the study of interspecific competition for space in elevationally replacing bird species (Robinson and Terborgh 1995, Jankowski, Robinson, and Levey 2010, Freeman 2016). Simulated heterospecific territory intrusions have shown that a response to heterospecific song by approaching the speaker is a signal of heterospecific aggression and that such aggressive interactions form an important element of interspecific competition at range boundaries (Grether et al. 2009,Peiman and Robinson 2010). Studies using song playbacks in birds have highlighted two recurring patterns in interspecific aggression between species; 1) interspecific aggression is often asymmetric suggesting one species is behaviorally dominant over the other (Robinson and Terborgh 1995, Jankowski, Robinson, and Levey 2010) and 2) in parapatric species, the intensity of aggressive responses is highest in the "contact zones" where the two species' ranges meet and diminishes away from the contact zone (Jankowski et al. 2013, McEntee 2014).

Research exploring the role of interspecific competition in setting species elevational range limits however, has seldom investigated species living in temperate climates. Instead, research has largely focused on tropical montane species which occur in relatively stable climates and resource regimes (Jankowski et al. 2013). Outside of the tropics, resident species in temperate habitats endure large temperature variation annually and resource availability varies significantly across seasons. This drives species to compete over different limiting resources in the breeding and non-breeding season (for e.g. food only in the winter and space only in the breeding season) as opposed to tropical

species which are territorial for longer periods and do not endure similarly large fluctuations in resource availability (Dhondt 2012). Furthermore, the little work done on elevational species replacement in temperate areas has focused on interactions involving long distance migrants or elevational migrants that only seasonally co-occur in their high elevation breeding grounds (Price 1991, Freeman and Montgomery 2015). Studies investigating competition across the year, over distinct resources are rare yet critical to understand the ecology of montane birds in temperate habitats. Resident species showing elevational replacement in temperate habitats represent important, understudied candidates for evaluating the possible significance of interspecific competition along elevational range boundaries.

Cinereous tits (*Parus cinereus,* formerly *Parus major cinereus*) and green-backed tits (*Parus monticolus*) are a pair of very closely related species (Johansson et al. 2013) that are both resident and show elevational replacement in the temperate Himalayas (Freeman 2015). They have significant ecological and morphological overlap (2.1) and throughout the Himalayas, the high elevation green-backed tit elevationally replaces the low-elevation cinereous tit. In the north-western Himalayas, along natural slopes, the two species have a narrow sympatric zone between 1300m-1400m where they replace each other at the ecotone of subtropical pines and *Quercus* (oak) forests (Whistler et al. 1949, Ali and Ripley 1983).

Human-modified habitats such as settlements and agricultural land are occupied by cinereous tits up to 1600m elevation in the Himalayas (Bhatt and Joshi 2011), well above the species' natural elevational distribution (Price et al. 2003) (Bhatt and Joshi 2011). Green-backed tits are found in human modified habitats in the non-breeding

season (winter) but almost exclusively use natural habitats (oak forests) in the breeding season (spring) (Bhatt and Joshi 2011). This pattern of distribution could have originated in two ways 1) high-elevation, human-associated cinereous tits compete with and displace green-backed tits from human-modified habitats in the breeding season or 2) cinereous tits occupy habitats vacated by green-backed tits. Resolving which species is competitively dominant can explain the importance of interspecific competition in driving the origins of the current pattern in distribution in particular and the importance of interspecific competition in driving avian elevational ranges in general.

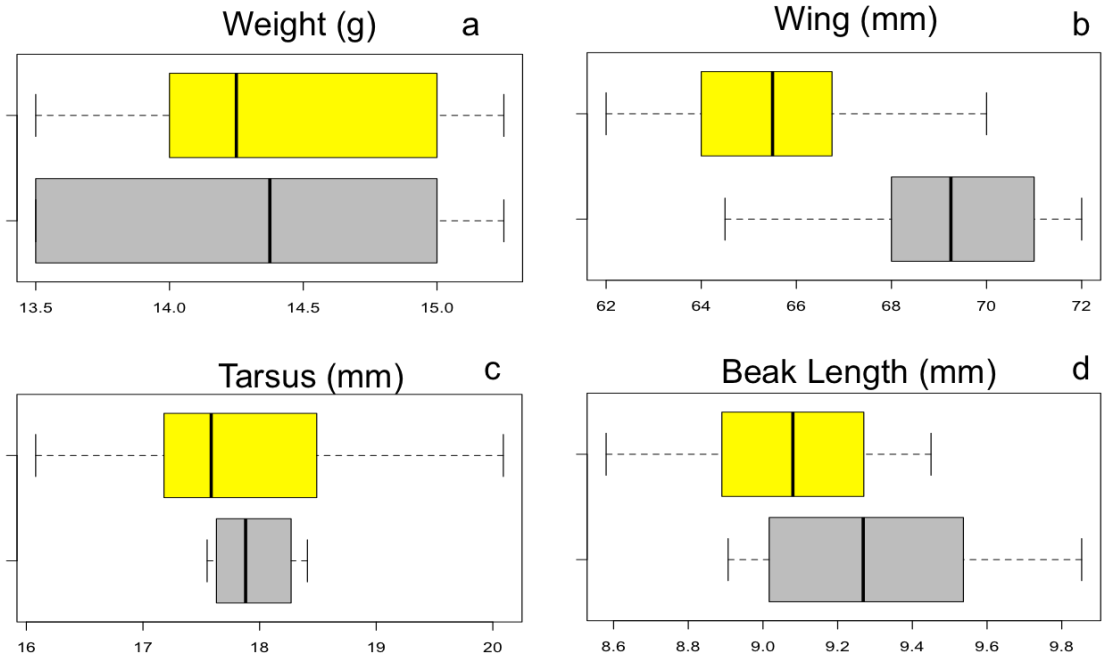

Figure 3.1: Comparison of key eco-morphological variables between male green-backed tits (light grey) and cinereous tits (dark grey) in a) Weight, b) Wing length, c) Tarsus length and d) Beak length in the overlap zone. None of the eco-morphological variables are statistically different.

We used song playback experiments in spring in sympatric (overlap) and allopatric (non-overlap) areas to test whether these species show heterospecific aggression highlighting competition for space in the overlap zone. Using feeder experiments in winter we tested whether the direction of heterospecific aggression over food in the non-breeding season is the same as that for space in the breeding season.

Materials and methods

Study sites

Behavioral experiments were conducted at three sites: (1) a low-elevation subtropical dipterocarp forest near Dehradun (30.2764°N, 77.9613°E, 600m ASL) where cinereous tits are allopatric, (2) the mid-elevational Mandal valley in the Kedarnath Wildlife Division (30.4574°N, 79. 2746°E, 1500m ASL) where cinereous and green-backed tits are sympatric, and (3) a high-elevation site at Bulkana also in the Kedarnath Wildlife Division (30.4770°N, 79.2100°E, 2800 ASL) where green-backed tits are allopatric. Song playback experiments, described below, were carried out in all three sites but feeder experiments were carried out only in the sympatric site.

Field methods and statistical analyses

a) Breeding season reciprocal song playback experiments

We recorded natural cinereous tit and green-backed tit songs when individuals were not involved in interactions with neighbors or as a response to playback using a Roland R-26 Recorder (44.1-kHz sampling rate, 16-bit encoding, WAV files) and a Sennheiser ME67 directional microphone at all three sites. In the playback experiments at each site we used

local conspecific recordings, and heterospecific recordings from the sympatric areas randomly choosing conspecific and heterospecific songs from among five to seven recordings. For the playback experiments at each site, we walked along trails and identified presumed territories of singing individuals for each species, marking each territory by tying a small ribbon to a low branch within that territory. Each playback consisted of 2 minutes of a control song (unrelated large, high elevation thrush (*Zoothera monticola*)) followed by 2 minutes of either conspecific or heterospecific songs. When doing heterospecific trials we always played heterospecific song first. In the case of a heterospecific response we did not conduct a conspecific trial on that day. If there was no response to heterospecific song, we did a conspecific song trial after a fifteen-minute break. We recorded three metrics of response to each song played in a trials 1) latency to response 2) latency to vocalizations and 3) distance approached to the speaker (Jankowski, Robinson, and Levey 2010, Freeman 2016). Due to field conditions like thick vegetation and other visual obstructions and loud environmental sounds (streams and rivers) we could only accurately quantify distance to speaker and used it as the response variable. An approach to less than 20m of the speaker was considered an aggressive response if the bird made direct flights towards the speaker. We only report responses to playback experiments conducted in the breeding season (15^{th} March- 30^{th} April 2014, 2015 and 2016) as neither species responded to conspecific or heterospecific song in the winter (non-breeding season) suggesting that neither species is strongly territorial in the winter.

Intraspecific competition is a pre-requisite to interspecific competition (A. A. Dhondt 2012), so first, we examined whether each species responded to conspecific song

at each location (sympatric or allopatric). This was the case in all sites. We then examined if response to conspecific song was more frequent and/or stronger than response to heterospecific song at each site. Finally, since heterospecific aggression is most commonly seen in areas of distributional overlap and diminishes away from the range boundary (Freeman 2016), we tested whether there was a difference in the frequency with which each species responded to heterospecific and conspecific playback across locations (sympatric and allopatric). As few cinereous tits responded to green-backed tit song in the sympatric site (n=3) and no green-backed tits responded to cinereous tits songs in allopatry, we could not compare the intensity of response (distance approached to the speaker) shown by the two species towards playback.

We compared species responses to conspecific and heterospecific song playbacks using 2×2 contingency table analyses with a χ^2 test using Yates correction. If any frequency of responses was less than five, we performed a two-tailed Fischer's exact test using Statistix 10 (Analysis software, Tallahassee, Florida). To test the hypothesis that response to conspecific song is stronger than response to heterospecific song we made four comparisons (see Table 3.2) thereby inflating the probability to find a difference by chance; similarly, to test the hypothesis that response in sympatry would differ from that in allopatry we again made four comparisons (see 3.2). Because in each case we tested response to playback four times, we report Holm-Bonferroni-corrected P-values for all results whose uncorrected P-value was <0.06 (Holm 1979)

b) Non-breeding season feeder experiments

We investigated the outcomes of competitive interactions over food (a putative limiting factor in the winter) by providing hanging feeders that excluded other sympatric non-parid species in two locations about 1.5 kilometers apart in villages where both species co-occur in the winter (January-February 2015). We performed 23 feeding trials to investigate competitive interactions between cinereous and green-backed tits at concentrated food sources. Feeder trials were conducted within the first hour after dawn when birds are most hungry (Pryke and Griffith 2006). From over 900 min of observations (~45 min/trial), we recorded the number of antagonistic interactions between one cinereous tit and one green-backed tit in which one of the individuals was displaced through a threat display, or physical attack (Prior and Weatherhead 1991)(Pryke and Griffith 2006). We inferred the sex of the birds based on the breadth of the breast stripe (Slagsvold 1993) or where possible knew an individual's sex based on prior handling and color bands placed on its legs .Whenever possible, we recorded the sex of the aggressor and of the displaced bird. We could not perform feeder experiments in the breeding season as the two species rarely co-occurred in the villages.

To test if one species more frequently won an interaction resulting in a displacement of the other species we calculated the exact P-value using a two-tailed "One Proportion test" in Statistix 10, whereby we tested if the proportion wins was significantly different from 0.5.

Results

Playback experiments during the breeding season

Green-backed tits showed heterospecific aggression significantly more often in sympatric than allopatric sites (P=0.028) (Figure 3.2d). Cinereous tits on the other hand, did not

show an exaggerated response to heterospecific song in the overlap zone (χ^2=0.384, P=0.5357, Figure 3.2b). Both species responded equally frequently to conspecific song across sites (green-backed tit χ 2=1.48, P=0.440, cinereous tits χ 2=3.769, P=0.10 Figure 3.2a and 3.2c). True to predictions in the literature, at each site both species responded more frequently to conspecific than heterospecific song (Table 3.1).

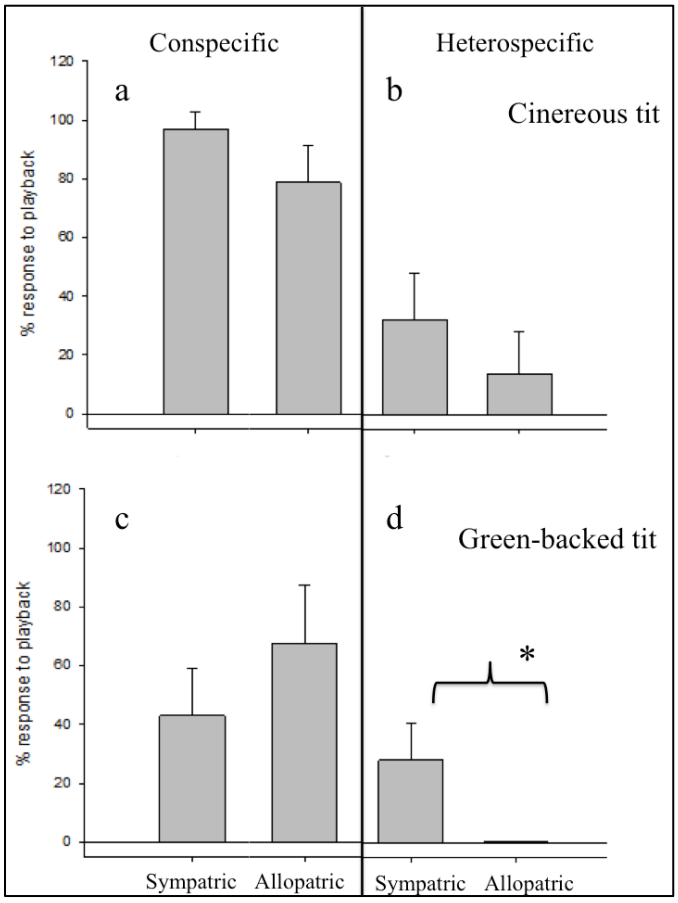

Figure 3.2: Comparison of frequency of response to conspecific and heterospecific playback experiments across sites in cinereous (a, b) and green-backed tits (c, d). Green-backed tits responded significantly more often to cinereous tit song in sympatric areas than in allopatric areas (d, P=0.001). In all other instances there was no significant difference in the frequency of response to conspecific or heterospecific song across sites (2a-2c). The error bars indicate 95% CI.

	Cinereous tit		Green-backed tit	
Location	Sympatric	Allopatric	Sympatric	Allopatric
Response	C>H	C>H	C>H	C>H
Resp /No Resp	(33/9), (3/19)	(31/1), (8/25)	(15/10), (14/36)	(17/4), (0/21)
χ^2	22.17	32.75	5.91	25.3
Df	1	1	1	1
P	0.000	0.000	0.015	0.00001

Table 3.1: Difference in frequency of response to conspecific (C) vs. heterospecific (H) playbacks in sympatric and allopatric areas in cinereous tits and green-backed tits. In all cases species replied more often to conspecific song than to heterospecific song (C > H). Numbers in brackets indicate number of playbacks in which birds responded / number of trials in which birds did not respond.

Feeder trials during the non-breeding season

Green-backed tits were behaviorally dominant over cinereous tits at feeders. At both experimental locations, roughly equal numbers of individuals of each species were typically present simultaneously, as parts of small single species flocks of 5–6 birds. Green-backed tits showed heterospecific aggression towards cinereous tits by displacing them from feeders significantly more frequently than expected by chance than vice versa (Table 3.2: 88 wins by green-backed tit versus 60 wins by cinereous tit P=0.0261). The interactions between these species were however more complex and, as in several other competing tit species of similar size governed by the sex and rank of the individual in the social hierarchy (Dhondt 1989, Lens 1996) . Using our observations of heterospecific interactions between individuals of known sex, green-backed tit males were the most

dominant: green-back-tit males won 37 of 45 (82%; P<0.0001) interactions involving a cinereous tit male and all 8 interactions with cinereous females (P=0.008). Cinereous tit males were dominant over green-backed tit females (34 wins out of 42 interactions – 80.9%; P=0.0001) (Table 3.2). In contrast cinereous tit females lost all 23 interactions with green-backed tits of either sex (P<0.0001).

Number of antagonistic interactions by sex				
	Heterospecific Male	Heterospecific Female	Unknown sex	Total
Green-backed tit (Male)	37	15	10	62
Green-backed tit (Female)	0	8	3	11
Green-backed tit (Unknown)	5	3	7	15
Total	**42**	**26**	**20**	**88**
Cinereous tit (Male)	8	34	3	45
Cinereous tit (Female)	0	0	0	0
Cinereous tit (Unknown)		5	10	15
Total	**8**	**39**	**13**	**60**

Table 3.2: Number of heterospecific antagonistic interactions seen at feeders between green-backed and cinereous tits with a breakup by sex of the aggressor (rows) and the displaced bird (columns).

Discussion

Our results suggest that unlike on tropical mountains, interspecific interactions among birds on temperate mountains likely change with season and are specific to resource limitation. Both playback and feeder experiments show that in sympatry green-backed tits are behaviorally dominant over cinereous tits. Both species show a weak or no response to heterospecific song in non-overlap areas, but only green-backed tits show an aggressive response to cinereous tit song where the two species co-occur. While green-backed tits are behaviorally dominant over cinereous tits both in the breeding and non-breeding season this competitive dominance does not seem to determine species' elevational distribution. Despite being competitively subordinate, cinereous tits might be expanding their elevational range by occupying the fast growing human-modified habitats vacated by green-backed tits in the sympatric zone. Interspecific competition might thus be less important than habitat in setting limits on elevational species distribution in temperate areas (Tingley et al. 2009).

Asymmetric responses in playback experiments, as seen in green-backed tits in sympatry, are a commonly documented pattern (McEntee 2014, Halfwerk et al. 2016). Green-backed tits, where they do not co-occur with cinereous tits do not respond to cinereous tit songs. Thus green-backed tit response to cinereous tit song in sympatry represents a learned response to the presence of heterospecific competitors. Through our feeder experiments, we could test the direction of heterospecific aggression in the winter when food is likely scarce. Aggression seen at feeders confirmed that green-backed tits are the behaviorally dominant species throughout the year. Although little literature beyond classical natural history exists on the habitat ecology of Himalayan birds, green-

backed tits are known to occur in significantly lower abundance in human modified habitats in allopatric areas (Bhatt and Joshi 2011). These habitats thus represent sub-optimal habitats for green-backed tits. Being behaviorally dominant, green-backed tits are however able to successfully invade human settlements occupied by the ecologically comparable cinereous tits when food is scarce in natural habitats in winter, outcompeting cinereous tits at concentrated food resources. When closely related species co-occur, subordinate species are predicted to be relegated to sub-optimal habitats due to competitive interactions (Murray Jr 1971). This is substantiated by the low abundance of cinereous tits outside human modified habitats which is likely driven by competitive interactions with green-backed tits.

Cinereous tits are a widespread generalist species and the sympatric site represents the elevational upper limit for the species' distribution in the western Himalayas. Although found commonly in forests at lower elevations in the same region (Singh 2000) cinereous tits are largely restricted to human habitations in the sympatric site and other locations at this elevation (Price et al. 2003, Bhatt and Joshi 2011). Urbanization is known to increase individual bird survival in harsh weather (Zuckerberg et al. 2011) and human modified habitats thus might facilitate cinereous tit survival at these elevations above their natural distribution which likely represent their physiological tolerance limits (Tingley et al. 2009). Despite being similar in size, cinereous tits are behaviorally subordinate to green-backed tits. The behavioral subordination in cinereous tits might be attributed to the physiological stress that they are exposed to at their elevational upper limit (Jankowski et al. 2013).

Our research on interspecific competition in elevationally replacing species has highlighted the importance of habitat in driving species distribution on temperate mountains. Human populations are increasing rapidly in the Himalayas which has led to large scale habitat conversions and deforestation in oak habitats (Pandit and Kumar 2013). Cinereous tits are found in human habitations up to about 200m above their natural distribution. The fact that green-backed tits are behaviorally dominant over cinereous tits in competitive interactions suggests that cinereous tits are able to expand their range upslope by occupying sub-optimal habitats vacated by green-backed tits. This finding is of conservation concern since the green-backed tit; a common high elevation species is possibly an indicator of the effect of habitat loss on Himalayan birds in general. By drastically altering habitats, anthropogenic changes might be transforming bird communities and species elevational distribution, diminishing the impact of mechanisms like interspecific competition at maintaining elevational ranges in the Himalayas.

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CHAPTER 4

Anthropogenic habitat change is driving upslope range expansion of a common low-elevation bird, the cinereous tit (*Parus cinereus*) in the western Himalayas.

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Abstract

Human populations are growing rapidly in the ecologically critical mid-elevations of the Himalayas causing extensive habitat loss as forests get converted to agricultural and pastoral land-use. Habitat alterations change local bird communities, favoring generalists and human-commensals. Yet we don't fully understand how habitat alterations affect elevational distribution of birds and the biological interactions that restrict species elevational ranges. We studied fine scale occupancy of two closely related elevationally replacing species, the cinereous tit (*Parus cinereus*) and the green-backed tit (*Parus monticolus*) in an elevational sympatric zone. Contrary to predictions in the literature our two-species conditional occupancy modeling demonstrates that interspecific competition does not drive the distribution of either species. Cinereous tit occupancy is highly associated with human-settlements suggesting that they have colonized areas above their natural distribution through human-commensalism. Green-backed tit occupancy on the other hand was associated with distance away from human settlements and areas with high tree-density. This suggests that cinereous tits are expanding their range upslope due to anthropogenic habitat changes while green-backed tit habitats are dwindling due to growing human populations in the western Himalayas.

Key words: Himalayas, anthropogenic habitat loss, interspecific competition, occupancy, range expansion, elevational distribution

Introduction

On mountains around the world, systematically changing ecological and environmental factors limit the distribution of most bird species to bands along the elevational gradient (Sekercioglu et al. 2008). Elevationally-limited species distribution and the consequent species turnover is the primary reason for the exceptional species richness seen in montane biotas (Graham et al. 2014). Several factors such as interspecific competition (Jankowski, Robinson, and Levey 2010), habitats (Terborgh 1985), and temperature (Tingley et al. 2009) are known to limit the elevational distribution of a species. Human altered habitats, through deforestation and conversion to other land-use types, is an extensively growing, increasingly prominent feature of montane ecosystems globally (Brooks et al. 1999). Located largely in the most populous countries in the world, the Himalayas a biodiversity hotspot (Myers et al. 2000), are facing exceptionally high levels of deforestation and habitat conversions (M. K. Pandit et al. 2007, Puyravaud, Davidar, and Laurance 2010). Yet, understanding how anthropogenic habitat change affects the elevational distribution of Himalayan birds is essential for biodiversity conservation in this region (Elsen et al. 2016).

At global scales, due to climate warming, low elevation species are predicted to expand/shift their distribution upslope (Freeman and Freeman 2014). At the local scale, anthropogenic modification of habitats is known to favor invasive species, commensals and generalists in the community (Croci, Butet, and Clergeau 2008, Lee et al. 2004, Shochat et al. 2010). Hence, habitat conversions and urbanization in the mountains might especially expedite and facilitate the expected up-slope expansion of low elevation

generalist and human commensal species. This in turn will result in habitat loss of specialist montane birds.

Mid-elevations on mountains globally have very high species richness (McCain 2009). The mid-elevations in the Himalayas are ecologically significant as they form the lower elevational limit for most montane species, are breeding grounds for summer migrants and the wintering habitats for several elevational and latitudinal winter migrants (Dixit, Joshi, and Barve 2016, Somveille et al. 2013, Price et al. 2011). Mid-elevations also represent regions with the most extensive and rapid rates of land-use change in the Himalayas with the burgeoning human population and are thus in dire need of conservation (Pandit and Kumar 2013, Pandit et al. 2007).

Historical information on Himalayan avian ecology helps us study changes in species elevational distributions. The green-backed tit (*Parus monticolus*) replaces the closely related, low elevation cinereous tit (*Parus cinereus*) (Johansson et al. 2013) throughout the Himalayas. In the Garhwal Himalayas (in the state of Uttarakhand, India), along natural slopes, the two species are known to replace each other at the ecotone of subtropical pines and *Quercus* (oak) forests between 1300m-1400m (Ali and Ripley 1983, Jerdon 1864, Whistler et al. 1949, Singh 2000). However, new records suggest that cinereous tits have expanded their elevational range upslope and are common in, but restricted to, the rapidly growing areas of human-modified habitats like settlements and adjacent agricultural habitats in the Himalayas while green-backed tits are common in natural habitats at the same elevations (Bhatt and Joshi 2011, Dixit, Joshi, and Barve 2016). This suggests that cinereous tits are either expanding by displacing green-backed

tits from human-modified habitats or are occupying habitats rendered sub-optimal through habitat modification and thus vacated by green-backed tits at these elevations.

Across a gradient of forest to human settlements, we use two species conditional occupancy models, (Richmond et al 2010), to test whether habitat or interspecific competition predict the fine scale habitat use patterns of these two species in sympatry in both winter and summer. We demonstrate that green-backed tits are not competitively subordinate to cinereous tits. In this altered zone of sympatry, the montane green-backed tits is dependent on natural forested habitats in the breeding season and use both forests and human-modified habitats in the winter. Cinereous tits on the other hand, only occupy the potentially sub-optimal human-modified habitats like settlements across the year. We thus present a case of anthropogenic habitat change driving the up-slope range expansion of a low-elevation human-commensal species. Although we modeled two species to minimize ecological differences between them, our results are relevant for several human commensal birds that might be expanding their range due to habitat conversion in the Himalayas.

Methods

Data collection

The study was carried out in three adjacent valleys in the Amrutganga catchment in the Kedarnath region of the western Himalayas (1540m-1580m). This area contains the highest elevational distributional limit of the cinereous tits and lower distributional limit of the green-backed tit in the Garhwal region. We surveyed the occupancy of these two species at 68 point-count stations at a distance of 100 m from each other along five

walking trails. The points spanned the full diversity of land-use practices in these valleys and in the region in general, including forest, forest-farmland edge, farmland and human habitation. We controlled for elevation with a maximum difference of 40m between the highest and the lowest point count station. We conducted five-minute point counts after an initial two-minute settle-down period following the observer's arrival at each station, and our data only included birds detected in a 20m radius around the observer (Ghosh-Harihar 2013, Pagen, Thompson III, and Burhans 2002, Verner 1985). Although we used both visual and aural cues for detection, more than 95% of the detections were visual. Each point was surveyed on seven different occasions in May 2014 (breeding season) and six occasions in Jan-February 2015 (non-breeding season) to generate detection/non-detection histories for the two species. At each survey site we also collected environmental covariates that potentially influence occupancy and detection probabilities for these species. At each pount-count station, we estimated tree density, tree species richness, average tree height, percentage canopy cover, shrub cover and grass cover. We also estimated the distance of each point to the nearest human settlement using the scale function in **Google Earth**.

Data Analyses

We used two species conditional occupancy models (Richmond et al 2010) to test various hypotheses to infer the influence of interspecific competition and habitat covariates on the occupancy and detection probabilities of cinereous and green-backed tits in summer and winter. Conditional occupancy and detection parameterizations in these models allow for the explicit modeling of the effects of a dominant species on the

occupancy and detection probabilities of the subordinate species. In addition, this model allows us to test how habitat factors influence the strength and direction of competition for space between the species pair of interest. The model consists of 8 principal parameters, representing the occupancy and detection probabilities of a dominant species 'A' and the conditional occupancy and detection probabilities of a subordinate species 'B

No	Parameter	Interpretation
1	Ψ^{A}	Probability of occupancy for A
2	Ψ^{BA}	Probability for occupancy for B given A is present
3	Ψ^{Ba}	Probability for occupancy for B given A is absent
4	p^{A}	Probability of detection for A given B absent
5	p^{B}	Probability of detection for B given A is absent
6	r^{A}	Probability of detection of A given both are present
7	r^{BA}	Probability of detection of B given both are present and A is detected
8	r^{Ba}	Probability of detection of B given both are present and A is not detected

Table 4.1 Parameters in the two species conditional occupancy model. A and B are the dominant and subordinate species, respectively

We took a two-step approach to model building as described in Richmond et al (2010). We first fit single-season single-species occupancy models (MacKenzie et al 2002) for each species for each season to determine the subset of covariates that best explain the detection probabilities for each species when the other is absent. Prior to fitting models we tested for correlations among covariates and retained only those variable pairs with an absolute coefficient correlation less than 0.4. We evaluated the effects of canopy cover and shrub cover on the detection probabilities of each species. Since a majority of the detections were based on visual cues, we hypothesized that

detection probability for both species should decline with increasing canopy and shrub cover. The detection model with the most support from the data, with the smallest AIC value among competing models were then used in the conditional occupancy models.

In order to determine the nature and direction of the competitive interactions between the species pair, we created two conditional occupancy (Richmond et al 2010) model sets for each season (summer and winter), by alternately modeling each species as dominant. Within each model set we estimated occupancy probabilities for the candidate dominant and subordinate species. These probabilities were then used to calculate a species interaction factor (SIF) defined by Richmond et al (2010) as

$$\text{SIF}=\frac{\Psi^{A}\Psi^{BA}}{\Psi^{A}(\Psi^{A}\Psi^{BA}+(1-\Psi^{A})\Psi^{Ba})}$$

Where Ψ is the occupancy probability of a species. Using a multi-model inference framework and the estimated SIF values, within each model set we tested for three species interaction scenarios.

1) The two species do not compete spatially and/ or behaviorally -i.e. Models with the following parameterization PsiBA=PsiBa and/or rBA=rBa should have higher support and have SIF=1. 2) The two species compete spatially and or behaviorally i.e. Models with the following parameterization should have higher support PsiBA<PsiBa or rBA<rBa and best supported models should have an SIF<1. 3) The two species share an ecological niche and occur together more often than expected by random chance. Models where psiBA > psiBa and SIF>1 should have maximum support if this hypothesis is true.

We also simultaneously tested the influence of habitat covariates on the occupancy probabilities of the two species by modeling the occupancy parameters (Ψ^{A}, Ψ^{BA}, Ψ^{Ba}) as linear functions of habitat covariates- tree density, distance to human

settlements. To further test the influence of human settlements on occupancy probabilities we created a binary variable (Settlement effect) by assigning a value of 1 (Settlement) to all points lying within a 70 m buffer around a human settlement and a value of 0 (Non-settlement) to any points lying outside it .We also tested for any interactive effects of tree density and settlements on occupancy probabilities of both species (Table 4.2). At this elevation, since they are known to occur largely in human settlements (Bhatt and Joshi 2011), we predicted that in both seasons occupancy probabilities for cinereous tits should be higher in settlement sites and lower in non-settlement sites and vice-versa for green-backed tits. We further predicted that occupancy probabilities for green-backed tits should increase in the summer and decrease in the winter as distance to human settlements increases. Since both species are cavity nesters, we predicted that cinereous tits occupancy probabilities should be higher in settlement sites with higher tree densities whereas for green-backed tits it should scale positively with tree density outside of settlements.

No	Species	Parameter	Tree Density	Distance to human settlement	Settlement (1)/ Non-settlement (0)	Shrub Cover	Canopy
1	Cinereous tit	Occupancy probability	na	-/-	+/+	na	na
2	Cinereous tit	Detection Probability	na	na	Na	-/-	-/-
3	GB tit	Occupancy probability	+/+	+/-	-/-	na	na
4	GB tit	Detection Probability	na	na	Na	-/-	-/-

Table 4.2: Hypothesized covariate effects on occupancy and detection probabilities of Cinereous and GB tit species + and - indicate direction of the slope of the covariate - parameter relationship in summer/winter.'na' indicated that the specific covariate effect was not tested.

Under each of the 4 competition parameterizations (PsiBA=PsiBa and rBA=rBa or rBA≠rBa; PsiBA≠PsiBa and rBA=rBa or rBA≠rBa) we fit a total of 20 covariate combination models (Table 4.3). Thus each of the four model sets comprised of 80 models. The single-season single-species models and the 2-species conditional occupancy models were fit using the R extension for program Mark 8.0 (White and Burnham 1999) –Rmark (). All parameters were estimated using a logit link function.

Covariates on Occupancy Probability	
Cinereous tit	**Green-backed tit**
Settlement	Tree density
Distance to Human Settlements	Settlement
Tree density -Settlement interaction	Distance to Human Settlements
No covariates (.)	Tree density -Settlement interaction
	No covariates (.)

Table 4.3: Covariate combinations used to model occupancy probabilities of both species For each of the six basic competition parameterizations we generated 21 covariate models by combining one covariate for cinereous tit with a covariate for green-backed tit.

Results

Detections and Naïve occupancy

In summer we detected cinereous and green-backed tits in 11 out of 68 sites. The two species were however detected together at only 1 site. In winter naïve occupancy probabilities for both species were higher. We detected cinereous tits in 19 of 68 sites whereas green-backed tits were detected in 31of the 68 sites. In winter the two species were detected together at 10 of the 68 sites.

Detection probability

Point estimates of detection probabilities for both species were higher in winter than in summer. In both seasons estimated detection probabilities for cinereous tits were higher than those of green-backed tits (Fig1). Detection probabilities for both species in both seasons were not influenced by any covariates, and models specifying uniform detection probabilities across sites and occasions 'intercept only model'' had the greatest support in both summer and winter. The complete model sets are provided in Appendix 1.

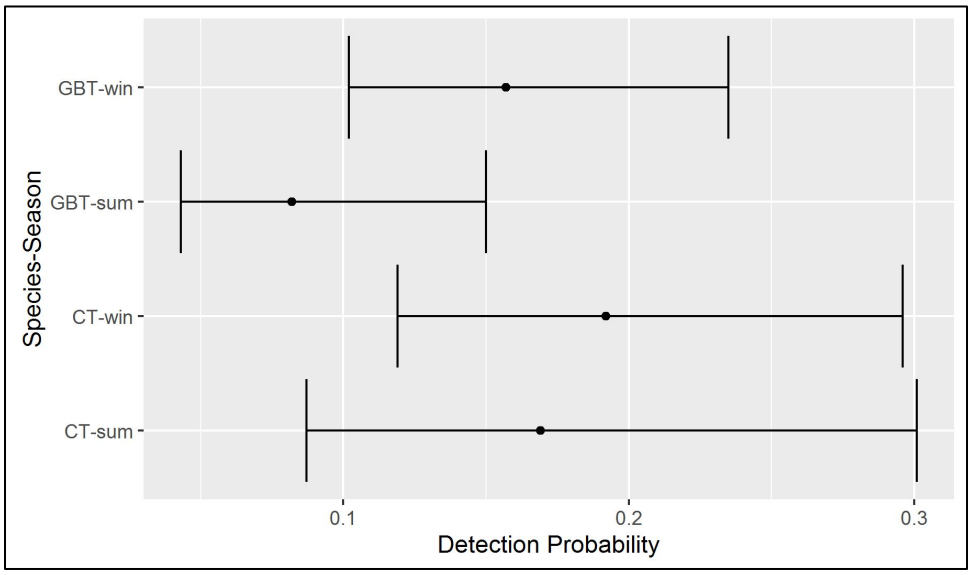

Figure 4.1: Detection probability estimates for cinereous tits (CT) and green-backed tits (GBT) in summer and winter

Interspecific competition

We built four candidate model-sets, each with 80 models (Appendix 2) to test the effect of interspecific competition on occupancy and detection probabilities of the two species. There was no evidence of competition for space between the two species in any of the four model sets. In all four model sets, model parameterizations where PsiBA=PsiBa had the highest model support. Since none of the 'top' models in any of the model sets had

high support we based our inferences on cumulative model weights for the different hypothesis of interest. Across all 4 model sets models specifying no competition for space between the two species had the highest cumulative weights (Table 4.4).

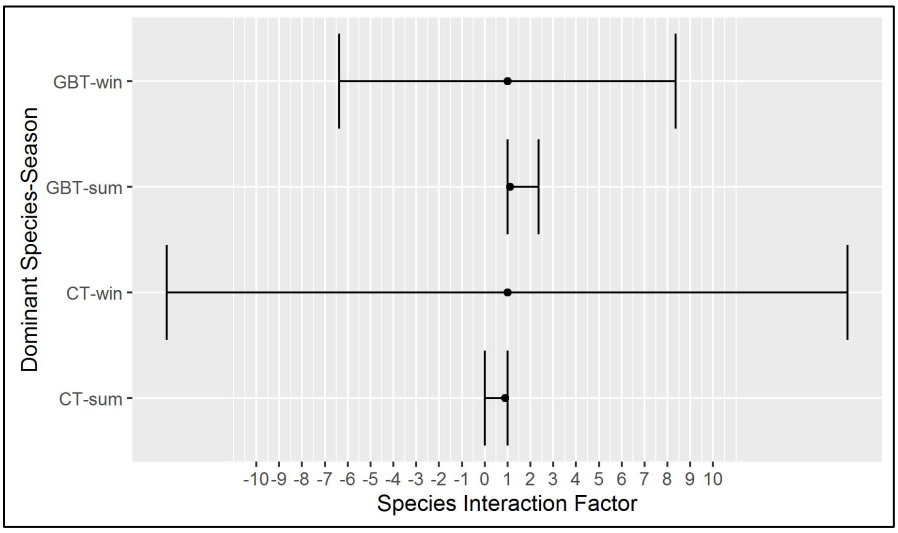

Figure 4.2: Modeled average estimates of species interaction factors for the four species-season model sets

Occupancy model	Detection Model	No of models evaluated with covariates		Cumulative model weight Cinereous tit dominant		Cumulative model weight Green-backed tit dominant	
		Summer	Winter	Summer	Winter	Summer	Winter
$\Psi^{BA} \neq \Psi^{Ba}$	$r^{Ba} \neq r^{Ba}$	20	20	0.118	0.051	0.2	0.02
	$r^{BA} = r^{Ba}$	20	20	0.059	0.093	0.031	0.11
TOTAL WEIGHT				0.177	0.144	0.231	0.13
$\Psi^{BA} = \Psi^{Ba}$	$r^{Ba} \neq r^{Ba}$	20	20	0.163	0.277	0.278	0.367
	$r^{BA} = r^{Ba}$	20	20	0.657	0.577	0.489	0.493
TOTAL WEIGHT				**0.82**	**0.854**	**0.767**	**0.86**

Table 4.4: Cumulative model weights for different competition hypotheses of interest from each of the four model sets. Models inferring no competition had significantly higher cumulative weight.

Model averaged estimates for SIF for all four models sets also supported the hypothesis of no competition for space between the two species. While model averaged point estimates of SIF (Fig 4.2.) from all four model sets were less than one, the confidence intervals for all estimates included 1, suggesting that the two species occur independently. Season specific model averaged estimated of occupancy probabilities for each species were consistent irrespective of dominant status. Model averaged estimates of occupancy probability for each species-season-dominance status combination are provided in Table 4.5.

Species	Dominance Status	Occupancy probability (Standard Error)	
		Summer	Winter
Cinereous tit	Dominant	0.306 (1.714)	0.925 (27.65)
	Subordinate	0.44 (2.13)	0.929 (38.73)
Green-backed tit	Dominant	0.92 (25.68)	0.736 (0.14)
	Subordinate	0.869 (1.54)	0.695 (1.298)

Table 4.5: Model averaged estimates of occupancy probabilities

In all four model sets the evidence for the absence of behavioral competitive effects was less ambiguous. In all model sets the 'top' model supported the hypothesis of no behavioral competitive effects, however models supporting behavioral competition were often the second or the third best supported models. (Appendix 2).

Determinants of Occupancy

Different sets of covariates influenced occupancy of the two species in summer and winter. Covariate effects however remained consistent irrespective of whether the species was modeled as dominant or subordinate. Tree density positively affected occupancy probabilities of green-backed tits in the winter whereas in the summer it scaled positively with distance away from human settlements (Fig 4.3a, 4.3b). Occupancy probabilities for cinereous tits in both seasons were affected by whether or not the site was within a settlement boundary. Settlement sites in both seasons were characterized by higher occupancy probabilities for cinereous tits. (Fig 4.4a, 4.4b). This effect is most pronounced in the winter season whereas in the summer there is considerably more uncertainty associated with the occupancy estimates.

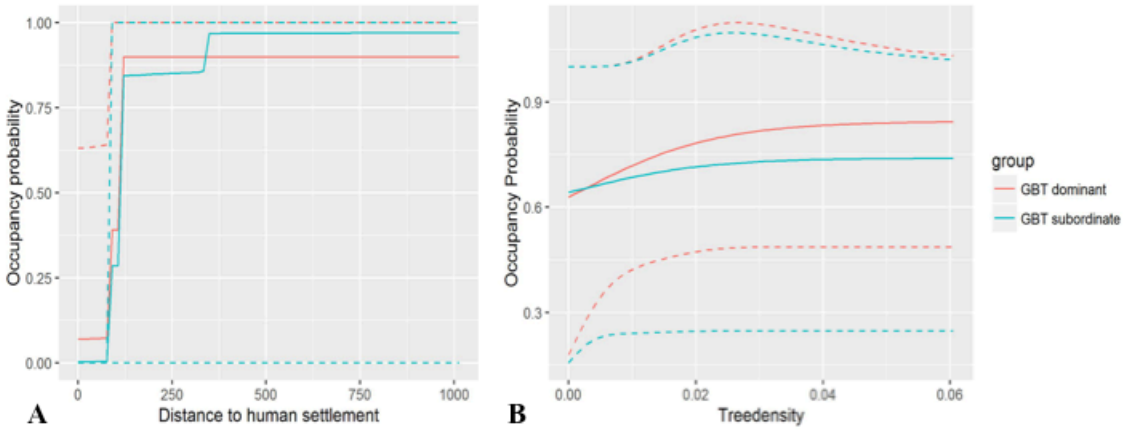

Figure 4.3: Relationship between (A) distance to human settlements in summer and (B) tree density in winter and green-backed tit occupancy probability

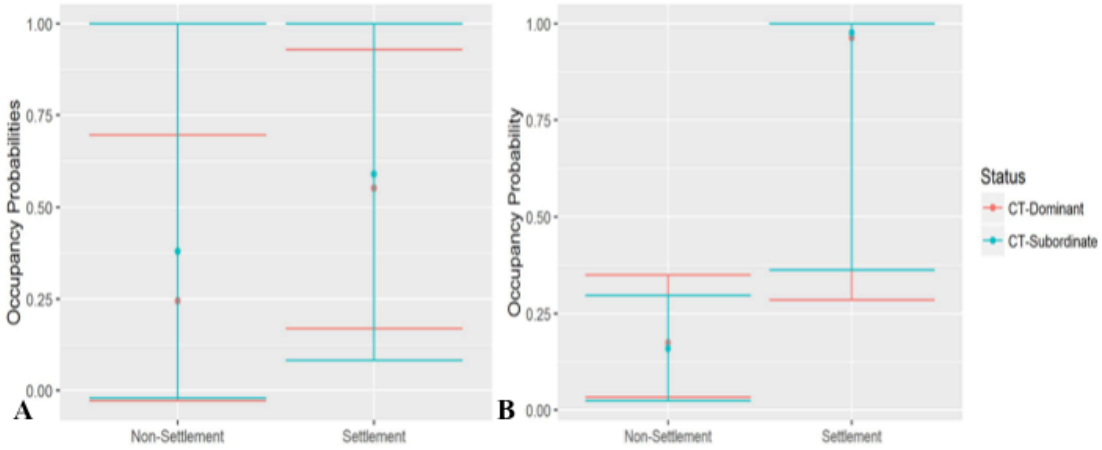

Figure 4.4: Cinereous tit occupancy probability at points within and outside human settlements in summer (A) and winter (B).

Discussion

At 1500m cinereous tits are strongly associated with human modified habitats throughout the year suggesting that they have expanded their distributional range upslope only after anthropogenic habitat changes. On the other hand, green-backed tits are associated with areas of high tree density and breed in areas away from human settlements which suggests that anthropogenic habitat changes create sub-optimal habitats for this species. Anthropogenic habitat changes, not interspecific competition, drive the patterns of site occupancy in this elevationally replacing species pair in the zone of sympatry. Our study thus highlights a new mechanism for generalist species to expand their distribution upslope through a close association with humans and human-modified habitats which is unsuitable for native montane species.

Cinereous tit occupancy is highly associated with human settlements in both seasons. Human settlements likely provide resources like consistent food resources and warm roosting sites in winter that alleviate the physiological costs of living at their

elevational upper distributional limit (Zuckerberg et al. 2011). In the breeding season (summer), higher cinereous tit occupancy in human settlement sites suggests that cinereous tits do not prefer to breed in oak habitats. Like cinereous tits, other species in the great tit (*Parus major*) complex are known to be associated with human settlements and human modified habitats at both elevational (Tibetan plateau) (Pfister 2001) and latitudinal range limits (Scandinavia) (Chamberlain et al. 2009, Jokimäki et al. 1996, Marzluff 2001). Hence, cinereous tits have likely colonized areas above their natural elevational distribution due to urbanization and habitat changes.

In both seasons, the green-backed tit is strongly associated with covariates of more natural habitats such as tree-density (winter) and distance away from human settlements (summer). This shows that green-backed tits generally prefer areas with low intensity of habitat alteration (Elsen et al. 2016) across the year. In the winter they tend to use areas with high tree density despite proximity to human settlements. In the summer (breeding season) it is found away from human settlements suggesting that forests are crucial habitats for breeding. Green-backed tits are a common forest bird in the western Himalayas (Ali and Ripley 1983). The same dwindling forests required by green-backed tits during the breeding season are also important breeding grounds for a suite of resident and migratory passerines at these mid-elevations in the Himalayas (Price et al. 2011). They are also an important over-wintering habitat for a large number of elevational migrants in the region (Dixit, Joshi, and Barve 2016). Thus the fragmentation and ultimate loss of forests in the mid elevations can lead to loss of the breeding habitats and crucial over-wintering habitats for a large number of Himalayan birds.

Interspecific competition has long been regarded as an important driver of elevational replacement of species (Terborgh and Weske 1975, Dhondt 2012) (Freeman 2015). Heterospecific aggression from low-elevation species is considered especially important in setting the lower range limits of montane species (Graham et al. 2009) (Jankowski, Robinson, and Levey 2010). Our conditional occupancy analyses show that competitive interactions with low-elevation cinereous tits have no influence on green-backed tit occupancy and vice-versa in both the breeding and non-breeding seasons and habitat alone explains the observed patterns of occupancy. Habitats and temperature have been shown to be more important in driving elevational distribution in the few other studies of temperate montane birds (Tingley et al. 2009). While the modeling approach we employed is appropriate to the questions we were interested in exploring, we concede that we were limited by a small sample size. In particular, we believe that the high uncertainty in estimates of occupancy and covariate effects and the derived parameter SIF may be a result of inadequate detections of the species despite considerable sampling effort. Future studies may benefit from using alternative approaches to detect these species such as the use of call playback experiments or mist-netting.

Elevational distribution like any dimension of a species distribution is driven by the interaction of several biotic and abiotic factors (Graham et al. 2014) and elevational distribution varies with latitude (Price et al. 2011). For example, the cinereous tit occurs at elevations higher than our study site (~2000m) in more northerly locations in the Himalayas, yet even there it is strongly associated with human modified habitats (Price et al. 2003) while green-backed tits occur in neighboring forests. Our study thus accurately

describes a broad pattern of human association and upslope range shift for the cinereous tit.

Anthropogenic habitat changes drastically alter natural habitats. This leads to significant changes in bird communities by selecting for specific life history and behavioral traits (Chamberlain et al. 2009, Croci, Butet, and Clergeau 2008, Stracey and Robinson 2012). Urbanization is known to affect species richness and species distribution along elevational gradients (Lee et al. 2004). Although we did not model their occupancy, our surveys indicate that similar to cinereous tits, other habitat generalist birds that

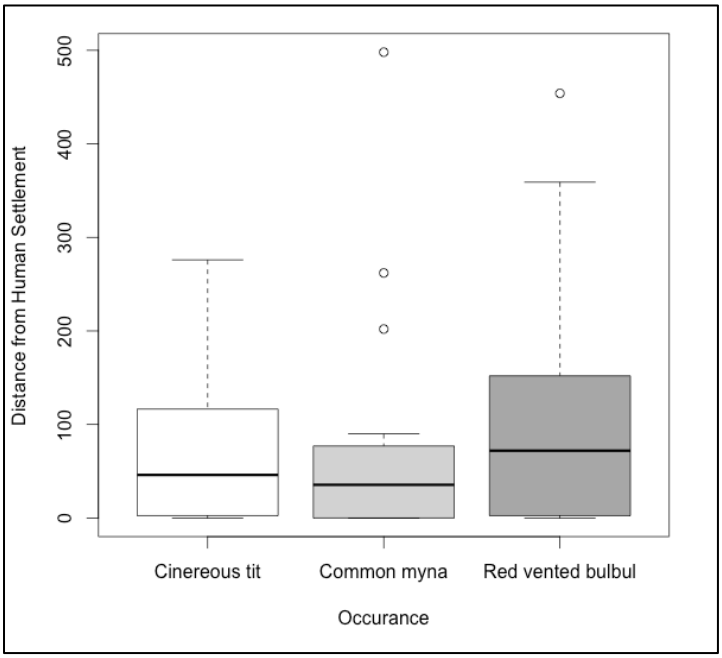

Figure 4.5: Distance of cinereous tit, common myna and red-vented bulbul detections from human settlements.

commonly associate with human modified habitats in addition to natural habitats at lower elevations such as the red-vented bulbul (*Pycnonotus cafer*), and common myna (*Acredotheres tristis*) were also only documented near human habitations in our study.

Species around the world are showing an upward range shift due to climate change (Freeman and Freeman 2014, Chen et al. 2011). Our findings suggest that upslope range shift might be expedited by anthropogenic habitat conversions for several generalist commensal species. Mid elevation forests are breeding grounds for several migratory species and are crucial over-wintering grounds for several elevational migrants. Our results demonstrate anthropogenic habitat modification influence distribution of common mid-elevation forest birds like the green-backed tit. Thus anthropogenic habitat loss is an emerging driver of elevational ranges for both low and high elevation species.

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CHAPTER 5

Sexual dimorphism in breast stripe width and beak eco-morphology in Himalayan Green-backed Tits (*Parus monticolus*)

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Key words: beak length, breast stripe, Green-backed Tit, Himalayas, sexual dimorphism

Abstract

Field data on sexual dimorphism is scant for most bird species. In this first field study of a western Himalayan population of Green-backed Tits (*Parus monticolus*), we demonstrate that breast stripe width is correlated with male condition in winter and is a reliable measure to sex this species in the wild. We also describe the sexual dimorphism in eco-morphological variables in the study population. Sexual dimorphism in beak morphology changed from winter to summer through male beaks shortening 4.5 % in summer with little difference in female beak measurements.

Introduction

Ecological and behavioral differences between males and females drive sexual dimorphism in birds. Research on the mechanisms driving sexual dimorphism (in morphology and plumage) has been crucial in the development of significant ecological and evolutionary theory (Owens and Hartley 1998). However, field data on sexual dimorphism remains scant for most passerines in the wild. Among passerines, the Great Tit (*Parus major*, family Paridae) has been used as a model species to investigate a suite of eco-evolutionary and behavioral hypotheses (Dhondt 2012). There is extensive research on breast stripe sexual dimorphism in Great Tits showing that breast stripes indicate male quality, social dominance and sex recognition (Figuerola and Senar 2000; Senar et al. 2014). The Green-backed Tit (*Parus monticolus*) is the sister taxon to the Great Tit species complex. It is a montane specialist of the Sino-Himalayan region (Johansson et al. 2013) and thus offers the exceptional opportunity to study trait evolution in sister taxa that inhabit distinct habitats. Like Great Tits, Green-backed Tits exhibit a

characteristic dark breast stripe that runs vertically from their throat down. Hofmann et al. (2007) showed that Green-backed Tits are dimorphic in their plumage under UV light. Sexes are known to differ in their breast stripes, however, there is no data on size and function of this trait in the wild (Gosler and Clement 2016).

Here, we present results from the first morphometric and molecular-based study on Green-backed Tits that quantifies intersexual differences in breast stripe width to explore the potential function of this trait. We also demonstrate seasonal variation in sexual dimorphism in eco-morphological traits in a western Himalayan population of this under-studied species.

Materials and Methods

Study site, sample collection and preparation

Birds were sampled using 6-9 m mistnets at several locations within the Mandal valley (30.44685° N 79.27328° E; ~1550 m A. S. L.), Kedarnath Wildlife Division in Uttarakhand, India. Each bird was sexed by measuring the breast stripe width and color. Black and broad breast striped birds were sexed as males and thin and ashy as females. Breast stripe width (at the base of the chest of the bird) was measured using a Standard Wing60/Universal Flush Wing Rule and mass using a Pesola 93010 Micro 30gx0.25s Spring Scale. 65 males and 48 females were sampled in winter (January-March 2014/2015) and 31 males and 18 females in summer (April-May 2014/2015). We measured beak (length, width, depth) and tarsus length using Fowler UltraCal Mark IV electronic calipers. Beak shape was calculated as the ratio between beak length and beak depth. Body condition was estimated as the ratio of the mass and the tarsus length of the

individual. We collected 20–40 μL of blood from the sub-brachial wing vein (never exceeding 1 % of the individual's body mass) of the birds. All samples were stored on FTA cards (Whatman®) of which 48 birds were sexed blindly using molecular markers following Griffiths et al. (1998). All birds were ringed and released at site after processing.

Statistical analysis

Breast stripe width

We performed an Analysis of Variance test (ANOVA) to quantify differences in breast stripe width between the sexes. We then performed a linear regression between both male breast stripe width and body condition (mass/tarsus) and raw male mass in winter (a period of resource limitation) to test if breast stripe is a potential signal of male quality. Due to small sample sizes, we could not perform this test on the summer data. To test if breast stripes were wider in larger males, we regressed breast stripes against tarsus length.

Sexual dimorphism and the eco-morphology of beaks

We first quantified intersexual differences in tarsus length which does not change with season/age in an individual. Since beak measurements are plastic across seasons (Gosler 1987), beak morphometrics were compared separately in winter and summer among sexes. In beak measurements that *did* change across seasons, we assessed if one sex changed significantly more using the interaction term between sex and season. In all comparisons, we used an ANOVA in the R programming language (R Development Core

Team 2015) after controlling for body size differences by dividing the variables by the tarsus length of the individual (Gosler 1987).

Results

Our genetic blind test confirmed that 97.9% (47/48) of the individuals were sexed correctly in the hand. Males had a significantly broader breast stripe (mean ± standard deviation 18.82 ± 4.06 mm) compared to females (10.66 ± 2.53 mm) ($F=47.61$, $P<0.001$, $N=48$, 76% broader) (Figure 5.1). Male breast stripe width was significantly correlated with winter raw mass ($r=0.44$, $P<0.001$) and marginally significantly with body condition ($r=0.38$, $P=0.054$), but not with tarsus length.

Figure 5.1: Sexual dimorphism in size and color of Green-backed Tit breast stripe: Top panel: Males have broad and black breast stripe; Bottom panel: females have thin ashy breast stripes. Males had a significantly broader breast stripe (mean ± standard deviation 18.82 ± 4.06 mm) compared to females (10.66 ± 2.53 mm) (F=47.61, P<0.001).

In winter male beaks were 4.2% longer (P<0.01) and 4.6% deeper (P<0.05) than females. Male beaks also had a different shape than females (P<0.001). In summer however, males and females did not differ in beak length or shape (P=0.33, P=0.14 respectively). Average male beak length in summer was 4.5% shorter than in winter while average female beak length was only 0.2% shorter in summer. Male beak length shortened significantly more than in females across seasons (Beak Length ~ Season*Sex, F=5.608, P<0.02, Figure 5.2). However, males did have significantly deeper beaks even in summer (P<0.001). (See Table 5.1 for detailed results).

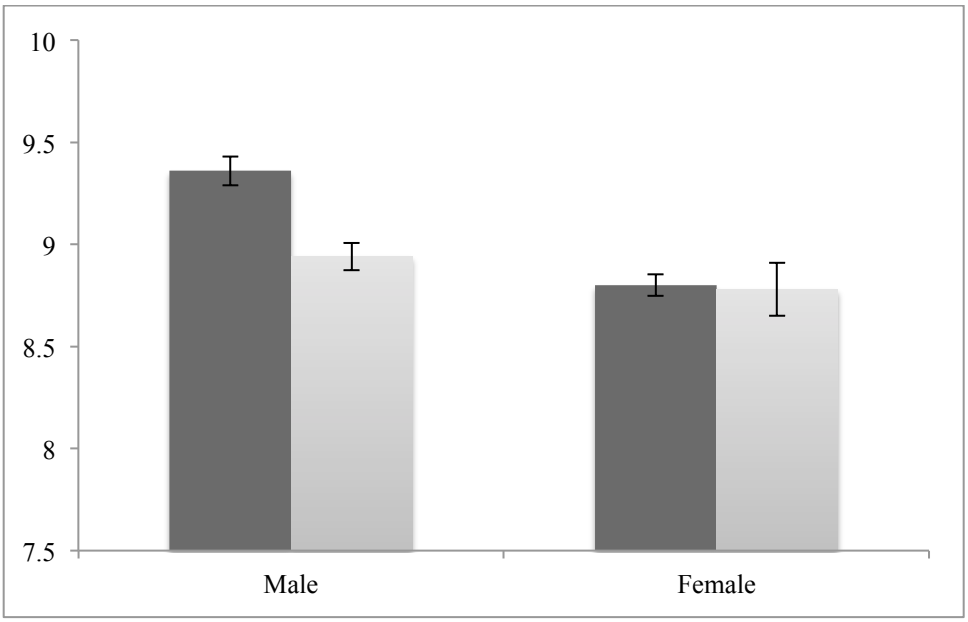

Figure 5.2: Change in male and female beak length (mm) across seasons. Male beaks shortened in length significantly more than female beaks from winter (dark grey) to summer (light grey) (Season*Sex interaction term, F=5.608, P<0.02). Female beak length did not change across seasons. Error bars indicate standard errors.

Discussion

Using morphometrics and molecular sexing, we confirm that Green-backed Tit males have a black and significantly broader breast stripe than females and hence this is a reliable measure to sex this species in the wild. In males, breast stripe width was correlated with both mass and condition in winter. Thus, breast stripe width might be indicative of quality in males of this species (Järvi and Bakken 1984). With the extensive literature on Great Tit breast stripes, Green-backed Tits represent an excellent system to study the parallel evolution of an easily measurable trait as a signal of quality and social status in two phylogenetically closely related species.

Measure (mm)	**Male (N=96)**	**Female (N=66)**	**Magnitude of Difference**
Tarsus	17.52 (0.96)	16.8 (0.84)	F= 4.11*** 4.2% larger
Winter	**(N=65)**	**(N=48)**	
Beak Length	9.36 (0.57)	8.80 (0.29)	F=5.63** 6%larger
Beak Width	4.31 (0.34)	4.40 (0.298)	F=0.89, P=0.367
Beak Height	4.03 (0.21)	3.85 (0.17)	F=2.86* 4.6% deeper
Beak Shape	2.32 (0.167)	2.182 (0.11)	F=8.83***
Summer	**(N=31)**	**(N=18)**	
Beak Length	8.94 (0.37)	8.78 (0.55)	F=0.58, P=0.33
Beak Width	4.64 (0.32)	4.44 (0.40)	F=0.42, P=0.67

Beak Height	4.16 (0.20)	3.99 (0.13)	F=7.76*** 4.3% deeper.
Beak Shape	1.87 (0.12)	1.96 (0.22)	F=1.5, P=0.14

Table 5.1: Morphological dimorphism in Green-backed Tit (*Parus monticolus*). Asterisks indicate significant difference with *=P<0.05, **=P<0.01 and ***=P<. 001. Numbers in parentheses are standard deviation. The significance of differences does not change when variables are controlled for size differences.

Green-backed Tits showed predictable sexual dimorphism in tarsus length. These differences were comparable to dimorphism reported in studies of Great Tits (Gosler 1987)(Dhondt et al. 1979). Similar to Gosler (1987), we found that sexual dimorphism in bill length and shape varies across seasons. While female beak length did not change with season, male beaks were 4.5 % shorter in summer (Table 5.1, Figure 5.2). This reduction in male beak length in summer is attributed to the disproportionately high wear on provisioning male beaks and relatively low wear on incubating female beaks in the breeding season (Matthysen et al. 1991). It is notable that seasonal changes in the dimorphism of beak morphology seen in Himalayan birds are comparable to European taxa. This result also suggests that differences in foraging ecology between male and female Green-backed Tits might be similar to differences in Great Tits. Studies quantifying foraging niches and diet can explain the selective pressures leading to these convergent differences.

The ecology of Himalayan birds is poorly understood and few studies have gone beyond studying the biogeography of species. Several Himalayan parids are closely related to well-studied species in Europe. This literature on European congeners can be used to study evolutionary ecology of morphological traits in congeneric Himalayan species inhabiting vastly different habitats.

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